



# JOURNAL OF AGRICULTURAL RESEARCH<sup>1</sup>

DEPARTMENT OF AGRICULTURE

VOL. VI

WASHINGTON, D. C., MAY 29, 1916

No. 9

## A WESTERN FIELDROT OF THE IRISH POTATO TUBER CAUSED BY *FUSARIUM RADICICOLA*

By O. A. PRATT,

Assistant Pathologist, Office of Cotton and Truck Disease Investigations,  
Bureau of Plant Industry

### INTRODUCTION

Tuber-rots of the Irish potato (*Solanum tuberosum*) which are common to the arid<sup>1</sup> West may be grouped into two classes: Storage-rots and field-rots. This paper is concerned only with certain rots attacking the potato tuber while growing in the field. From the tuber-rots under discussion, the fungus *Fusarium radicicola* Wollenw. was isolated. Carpenter<sup>2</sup> in 1915 demonstrated that *F. radicicola* could, under laboratory conditions, cause decays in potato tubers similar in every way to these rots. His experiments, however, were conducted wholly in the laboratories of the Department of Agriculture, in Washington, D. C. It was therefore thought practicable to present this paper, which gives the results of experiments performed under field conditions in the irrigated West. These experiments substantiate the results obtained by Carpenter and further establish the relationship of *F. radicicola* to the field tuber-rots under consideration.

### THE DISEASE

Under the head of fieldrot are considered several types of decay occurring in potato tubers while yet in the field—a stem-end rot, a lenticel rot, and a rot proceeding from eye infections. Eye infections in the field are not as common as stem-end and lenticel infections. These types of rot are known as “stem-end rot,” “field dryrot,” or “blackrot.” The name “blackrot” best describes them, for the decayed tissues are nearly black in color when the tubers are taken from the field. The rot may be further described as a comparatively dry rot, dark to nearly black in color, proceeding from the stem end, lenticels, and occasionally from the eyes of the tuber. The decay is first recognized by the blackened, sunken appearance of the stem end, or, in the case of lenticel and eye

<sup>1</sup> The observations and experiments set forth in this paper were confined principally to southern Idaho.

<sup>2</sup> Carpenter, C. W. Some potato tuber-rots caused by species of *Fusarium*. *In Jour. Agr. Research*, v. 5, no. 5, p. 183-210, pl. A-B (col.), 14-19. 1915.

infections, by the blackened, more or less sunken spots on the surface of the tuber. Tubers collected in a commercial potato field and infected in this manner are shown in Plate XXXIV, figures 1 to 6. This black color is lost in part as the infection becomes older, the infected tissues taking on various shades from nearly black to sepia brown. In connection with the stem-end rot, the fungus often proceeds down the vascular tissue, killing and blackening the network of bundles. Figures 5 and 6 in Plate XXXIV show sections of a tuber infected in this manner. Often it is possible to break away the cortical tissues and lay bare the blackened network. Lenticel infection proceeds outward in all directions from the point of infection and may or may not extend down to the main vascular system. Very frequently in the case of eye infections the vascular strand connecting the eye with the main vascular system is blackened, but it is seldom that such infection extends far into the main vascular ring. Blackrot is confined principally to potatoes of the Idaho Rural, Pearl, and other round types.

Closely related to the blackrot of potatoes of the round types is a jelly-end rot attacking principally varieties of the Burbank group. Jelly-end-infected tubers of the Netted Gem variety are shown in Plate XXXV, figures 1 to 3. The jelly-end rot of the Burbank group differs from the blackrot of round types of potatoes in that it is a softrot, light to dark brown in color, while the blackrot is a comparatively dry rot, black or nearly black in color. Jelly-end rot may be described as a soft, wet rot of the tubers proceeding from the stem end downward through the tuber attacking all tissues but apparently advancing somewhat more rapidly through the vascular bundles. Examination of tubers infected with jelly-end rot, however, often reveals no perceptible discoloration of the vascular tissue below the line of the rot in the other tissues. As the decay becomes older, the stem end becomes somewhat shriveled and dried, often closely resembling the type of decay caused in storage by *F. trichothecioides* Wollenw.<sup>1</sup> Lenticel and eye infections are seldom found in connection with the jelly-end rot of the Burbank group.

Occasionally a softrot of the seed end is also found. A Netted Gem tuber infected at both the seed end and the stem end is shown in Plate XXXV, figure 1. *F. radicicola* was isolated from both ends of this tuber. There was apparently no infection in the vascular tissues connecting the two regions of decay.

At first it was thought that the jelly-end rot of the Burbank group and the blackrot of round types of potatoes were two distinct diseases, but inoculations made in 1914 into the stem ends of Netted Gem and Idaho Rural tubers with *F. radicicola* led to the belief that they might be caused by the same organism. Material collected in the field, whether jelly-end rot or blackrot, when placed in a moist chamber for a few days

<sup>1</sup> Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by *Fusarium trichothecioides*, Wollenw. *In Jour. Washington Acad. Sci.*, v. 1, no. 6, p. 146-152, illus. 1912.

usually showed tufts of *F. radiculicola*. Infected tubers of Idaho Rural potatoes kept 10 days in a moist chamber at room temperature are shown in Plate XXXV, figures 4 and 5. Tufts of *F. radiculicola* have appeared. Inoculations in 1915 left no doubt in the writer's mind that *F. radiculicola* was capable of causing both types of rot.

#### DISTRIBUTION AND ECONOMIC IMPORTANCE

*F. radiculicola* is apparently widely distributed. Wollenweber<sup>1</sup> states that its habitat is "on partly decayed tubers and roots of plants, such as *Solanum tuberosum* in Europe and America (collected by Wollenweber) and *Ipomoea batatas* in the United States of America (collected by Harter and Field)." Carpenter<sup>2</sup> makes the following statement as to its habitat: "On partly decayed tubers and roots of plants. Cause of potato dryrot and jelly-end rot. Identified from the following: *Ipomoea batatas* (collected by Mr. L. L. Harter); *Musa sapientum* (collected by Mr. S. F. Ashby, Jamaica, Porto Rico); *Cucumis sativus* (collected by Mr. F. V. Rand, West Haven, Conn.); soil (collected by Mr. F. C. Werkenthin, Austin, Tex.)."

The writer has isolated *F. radiculicola* from the roots of poplar trees (*Populus deltoides*) at Jerome, Idaho, where he found it associated with crownrot. The fact that the fungus appears on potato tubers when disease-free seed potatoes are planted on raw desert lands suggests that it may be well distributed throughout the desert soils. Orton<sup>3</sup> in 1909 reported jelly-end rot of potatoes from the San Joaquin Valley, in California.

*F. radiculicola* has been reported on potatoes from Idaho, Oregon, and California by Wollenweber<sup>4</sup> and from Idaho, Oregon, California, Nevada, Mississippi, New York, Virginia, and the District of Columbia by Carpenter.<sup>5</sup> The writer has isolated this fungus from decayed potato tubers from the following localities in Idaho: Idaho Falls, Blackfoot, Aberdeen, Rupert, Murtaugh, Twin Falls, Filer, Kimberly, Jerome, Wendell, Gooding, King Hill, and Caldwell, and has observed the rot in potato fields in many other localities in the State. The disease apparently appears at its worst under dry-land-farming conditions and in raw desert land planted to potatoes before having been in other crops. On comparing rotted tubers collected by himself in Idaho with specimens sent to the Department of Agriculture from California and Oregon he was convinced that the rots were of one and the same nature. He has also observed rots identical in outward appearance with those found in Idaho, in Portland, Oreg., Seattle, Wash., and British Columbia.

<sup>1</sup> Wollenweber, H. W. Identification of species of *Fusarium* occurring on the sweet potato, *Ipomoea batatas*. In Jour. Agr. Research, v. 2, no. 4, p. 257. 1914.

<sup>2</sup> Carpenter, C. W. Op cit., p. 206.

<sup>3</sup> Orton, W. A. Potato diseases in San Joaquin County, Cal. U. S. Dept. Agr., Bur. Plant Indus. Circ. 23, 14 p. 1909.

<sup>4</sup> Wollenweber, H. W. Op. cit.

<sup>5</sup> Carpenter, C. W. Op. cit.

In the irrigated portions of Idaho the economic importance of the disease has varied greatly from year to year. In 1913 the writer was usually able to find only an occasional rotted tuber in any one commercial field. In a few fields which had been planted on raw desert land and poorly cared for he found as high as 80 per cent of the tubers infected with stem-end blackrot and lenticel rot. The year 1914 might be called an epidemic year. In one 50-acre field of Netted Gems near Jerome, Idaho, he found as high as 40 per cent of the crop infected with jelly-end rot. Similar conditions were observed in many other fields in the irrigated portions of southern Idaho. Stem-end blackrot and lenticel rot were also found very abundant in the fields of Idaho Rurals. It is significant that in 1914 a freeze occurred in June which killed the vines to the ground, the plants coming up anew and producing a crop. Often the origin of infection could be traced from the frozen tip of the vine down through the stem to the infected tubers. Although infected tubers were found in most of the commercial fields visited in 1915, the disease this year was of slight importance.

#### EXPERIMENTAL WORK

##### PRELIMINARY EXPERIMENT IN 1914

In the fall of 1914 ten Idaho Rural tubers and ten Netted Gem tubers were disinfected by dipping in formaldehyde and were punctured at the stem end with a needle carrying spores from a culture of *F. radicicola* which had been isolated from a potato tuber infected with blackrot. After inoculation the tubers were placed in moist chambers, where they remained for something over a month. An examination of the tubers showed that infection had been produced in every tuber inoculated. The infection in the Idaho Rurals was similar in all respects to the blackrot occurring in the field. The infection in the Netted Gems was not quite so dark in color as that produced in the Idaho Rurals and resembled certain stages of jelly-end rot collected in the field. No checks were prepared.

##### LABORATORY EXPERIMENTS IN 1915

On August 6, young and apparently healthy potato tubers of the Netted Gem and Idaho Rural varieties were selected, carefully washed, and disinfected in a solution of formaldehyde (1:240). After disinfection the tubers were dried and inoculated with *F. radicicola*. The methods of inoculation were as follows: (1) By spraying with a spore suspension; (2) by wounding the tubers with a needle bearing spores; and (3) by dipping the broken stolon ends in a spore suspension. In method 3 the tubers were taken from the field with their stolons attached. After disinfection each stolon was broken off afresh at from 1 to 2 inches from its junction with the tuber and inoculated as stated in the foregoing.

Fifty tubers each of Idaho Rural and Netted Gem, respectively, were inoculated by methods 1 and 2, and twenty-five tubers each of Idaho Rural and Netted Gem were inoculated by method 3. Checks on each experiment were prepared in the same manner, except that in method 1 the tubers were sprayed with sterile water, in method 2 the tubers were wounded with a sterile needle, and in method 3 the broken stolon ends were dipped in sterile water. Inoculated tubers and checks were placed in moist chambers and put in the culture room of the Experiment Station laboratory. During the course of these experiments the culture-room temperature varied from a minimum of 26° to a maximum of 29° C. Temperatures were taken daily at 8.30 a. m. and 5.30 p. m. After a month the tubers were examined. Table I gives a summary of the experiments and the number of tubers found infected.

TABLE I.—Summary and results of laboratory inoculations of *Solanum tuberosum*.

Method No.	Method of inoculation and parts inoculated.	Variety.	Number of tubers inoculated.	Number of tubers infected.
1	Tubers sprayed with suspension of spores . . . . .	Netted Gem . . . . .	50	48
		Idaho Rural . . . . .	50	50
		Netted Gem . . . . .	50	0
		Idaho Rural . . . . .	50	0
2	Tubers punctured with inoculated needle at stem end.	Netted Gem . . . . .	50	50
		Idaho Rural . . . . .	50	50
		Netted Gem . . . . .	50	0
		Idaho Rural . . . . .	50	0
3	Tubers; stem end punctured with sterile needle.	Idaho Rural . . . . .	25	25
		Netted Gem . . . . .	25	19
		Idaho Rural . . . . .	25	0
		Netted Gem . . . . .	25	0

Of the 50 Netted Gem tubers sprayed with the spore suspension, 48 showed infection. Stem-end infection was present in each of the inoculated tubers. Lenticel infections were present on most of the tubers, and eye infections were also found. Every Idaho Rural tuber sprayed with the spore suspension showed infection at the stem end. The majority showed lenticel infections and several showed eye infections. Lenticel infections, induced by spraying with the spore suspension, are shown in Plate XXXVI, figure 3. In figure 4 of Plate XXXVI is shown the same tuber after remaining several days longer in the moist chamber. Tufts of *F. radicola* have appeared over the surface of the decayed areas.

A stem-end infection of an Idaho Rural tuber sprayed with the spore suspension is shown in Plate XXXVI, figure 5. Every tuber, whether Netted Gem or Idaho Rural, developed infection when punctured at the stem end with a needle carrying the spores of the fungus. Decays induced in this manner are shown on Plate XXXVI, figures 1 and 2. Twenty-five stem-end tuber infections resulted from the inoculation of the broken stolon ends in the Netted Gems, and 19 in the Idaho Rurals. The decay resulting from this method of inoculation was similar in every

way to that produced by the other methods. A stem-end infection resulting from the inoculation of the broken stolon end under laboratory conditions is shown in Plate XXXVI, figure 6. In Plate XXXVI, figure 7, is shown an Idaho Rural tuber cut to expose the blackening of the vascular tissue which resulted from the inoculation of the tuber stolon. None of the checks were infected. The fungus was recovered from the decayed tissues each time the attempt was made.

#### EXPERIMENTS IN THE FIELD IN 1915

On August 11, in a plot in which disease-free Idaho Rural and Netted Gem seed potatoes had been planted, apparently healthy potato plants were selected. The soil was removed from around the plants in such a manner as to expose the tubers without disturbing their position. Three growing tubers under each plant were then inoculated with *F. radicola*, after which the soil was replaced, care being exercised to place moist soil next to the tubers. The methods of inoculation were, respectively, as follows: (1) By spraying the tubers with a spore suspension; (2) by wounding each tuber stolon with a needle bearing spores at from 1 to 2 inches from its junction with the tuber; (3) by wounding the upper surface of each tuber with a needle bearing spores, and (4) by puncturing each tuber at the stem end with a spore-bearing needle. Ten plants each of Idaho Rural and Netted Gem potatoes were used in each experiment. As a check on each experiment, a similar number of apparently healthy Idaho Rural and Netted Gem plants were selected and a similar number of growing tubers treated in the same manner, except that in the case of experiment 1 the tubers were sprayed with sterile water, and in numbers 2, 3, and 4 a sterile needle was used in place of a spore-bearing needle.

A fifth experiment was set up in which 10 apparently healthy Idaho Rural and 10 apparently healthy Netted Gem plants, growing in the same plot with those employed in the four experiments just described, were used. In this experiment, the stem of each plant was punctured at the crown with a needle carrying spores of *F. radicola*. Checks were prepared in the same manner, except that the stem of each plant was punctured with a sterile needle.

The soil of the plot in which these experiments were made was very dry and no irrigation water could be applied after the inoculations were made. During the course of the experiments (August 11 to September 6) the minimum soil temperature recorded was 66° and the maximum 84° F. The soil temperature was taken at a depth at which the potato tubers were found lying by burying the bulb of a soil thermograph under a potato plant. A little less than a month after making the inoculations an examination of all the plants was made. Table II gives a summary of the experiments and the results obtained from inoculating growing potato plants and tubers with *F. radicola*.

TABLE II.—Summary and results of inoculating growing potato plants and tubers with *Fusarium radiclecola*

Ex- per- iment No.	Method of inoculation.	Variety.	Number of inocu- lations.	Number of tubers infected.
1	Tubers sprayed with suspension of spores.....	Idaho Rural.....	30	15
	Check. Tubers sprayed with sterile water.....	Netted Gem.....	30	17
		Idaho Rural.....	30	0
2	Tuber stolons punctured with inoculated needle.....	Netted Gem.....	30	0
		Idaho Rural.....	30	27
	Check. Tuber stolons punctured with sterile needle.....	Idaho Rural.....	30	24
3	Tubers punctured with inoculated needle.....	Netted Gem.....	30	0
		Idaho Rural.....	30	30
	Check. Tubers punctured with sterile needle.....	Idaho Rural.....	30	0
4	Tubers punctured at stem end with inoculated needle.....	Netted Gem.....	30	0
		Idaho Rural.....	30	30
	Check. Tubers punctured at stem end with sterile needle.....	Idaho Rural.....	30	0
5	Stem of plant punctured at crown with inoculated needle.....	Netted Gem.....	30	0
		Idaho Rural.....	10	0
	Check. Plant stem punctured at crown with sterile needle.....	Idaho Rural.....	10	0
		Netted Gem.....	10	0

Of the 30 Idaho Rural tubers sprayed, 15 showed infection with stem-end and lenticel rot. Of the 30 Netted Gem tubers sprayed, 17 showed stem-end rot. Lenticel rot did not occur on all of the Netted Gem tubers and where it did occur the infections were very slight. The thicker skin of the Netted Gem probably renders it more resistant to fungus attacks than the Idaho Rural. The failure of a part of the sprayed tubers to develop infection can probably be attributed to the extremely dry condition of the soil. Infections resulting from spraying the growing tubers with a suspension of the spores of *F. radiclecola* are shown in Plate XXXVI, figures 1 to 4. In figure 4, Plate XXXVI, is shown an eye infection which has extended down into the vascular system. *F. radiclecola* was recovered from the discolored vascular tissue of this tuber. None of the checks showed any infection. Twenty-seven Idaho Rural tubers infected with stem-end rot resulted from the puncturing of the 30 tuber stolons. The three which failed to develop infection were under the same plant. Twenty-three of the Netted Gem tubers whose stolons were inoculated showed stem-end infection. Seven showed no evidence of infection in the tubers, though the stolons were black and dead up to within about one-eighth of an inch of their juncture with the tubers. Where infection in the tuber was found the line of infection could easily be traced down the stolon from the point of inoculation into the tuber.

Tuber infections resulting from the inoculation of the stolons in the field are shown in Plate XXXVII, figures 5 to 8. Both stem-end rot and vascular infection are shown. Figure 8, Plate XXXVII, represents a Netted Gem tuber with stem-end infection resulting from the inoculation of the

stolon. The rot in this case was nearly black in color, soft, and resembled the earlier stages of the jelly-end rot often found in commercial fields. Vascular infection also developed in this tuber. The fungus was recovered from all infected tissues whenever the attempt was made. None of the checks were infected. Infection resulted in all cases where tubers were punctured with a needle carrying the spores of the fungus. None of the checks were infected. In the case of the checks the punctures could be seen easily but were healed over in each case. The inoculations made into the stems of potato plants failed to give very decisive results. In each case a blackening of the tissue adjacent to the puncture was observed. This blackening extended up and down from the point of puncture for from one-eighth to one-half an inch and in most cases also extended into the pith.

#### BLACKROT

The infections, whether at the stem end, at the lenticels, or at the eyes, produced by the artificial inoculation of Idaho Rural tubers with *F. radicola*, could not be distinguished in any way from the infections on decayed tubers collected in the commercial fields. The infections resulting from the inoculation of growing tubers in the station plots when final examination was made were not as deep or as far advanced as many infections occurring naturally in the field, but this can easily be explained by the late date at which the inoculations were made. In fact, at the time the inoculations were made, tubers with well-advanced decay were being found in commercial fields. On the other hand, tubers with decay no farther advanced than that resulting from the inoculations have often been found in the field late in the season. In every case where an attempt was made, the fungus was recovered.

Tubers infected by inoculation in the field, by spraying with the spore suspension, by the puncture of the tuber with an inoculating needle, and by puncture of the tuber stolons, were placed in moist chambers, and in each case, after a few days, tufts of *F. radicola* appeared. Blackrot-infected tubers in commercial fields, after being kept in a moist chamber from 3 to 10 days at temperatures ranging from 65° to 75° F., invariably threw out tufts of this fungus (Pl. XXXV, fig. 4, 5). Isolations made from the cortical and medullary tissues of blackrot-infected tubers have never yielded any fungus other than *F. radicola*, which could be considered as the cause of the disease. Isolations made from stem-end blackrot-infected Idaho Rurals, Pearls, and other round types of potatoes have occasionally yielded *F. oxysporum*, especially when the culture was made from or near the vascular tissue. The failure to obtain *F. oxysporum* from lenticel and eye infections of tubers collected in commercial fields leads the writer to conclude that when *F. oxysporum* is found in stem-end infections it probably entered as a vascular parasite, independ-

ent of *F. radicola*. *F. oxysporum* has never been found in connection with the stem-end blackrot of western potatoes to the exclusion of *F. radicola*.

Fully 50 per cent of all cultures made from the decayed cortical and medullary tissues of tubers infected with stem-end and lenticel rot have remained sterile. This may have been due to improper cultural conditions, but it is believed that the discoloration of the tuber tissue often extends some distance beyond the point actually reached by the invading fungus. Stem-end blackrot-infected tubers often show a black net necrosis. Isolations made from the black network of bundles, if made some distance below the stem end, often fail to reveal any fungus. On the other hand, many such cultures have revealed *F. radicola*, and occasionally both *F. radicola* and *F. oxysporum*. That *F. radicola* is capable of causing the blackened net, as well as the stem-end blackrot, is fully demonstrated by the results of artificial inoculations Pl. XXXVI, fig. 7, and Pl. XXXVII, fig. 6, 8), though the fungus may not always be present throughout the entire length of the blackened bundle area.

#### JELLY-END ROT

Whenever the inoculation of Netted Gem tubers took effect at the stem end, an infection typical of certain types of jelly-end rot found in the commercial fields was produced. In the moist chamber under laboratory conditions infections at the stem end induced by puncturing the tubers, by spraying with a spore suspension, or by puncture of the stolons with an inoculating needle were fairly typical of the advanced stages of jelly-end rot, being soft and watery. Under field conditions, infections at the stem end induced by spraying the tubers with the spore suspension, by puncturing with an inoculating needle, or by the inoculation of the stolons were in no case as pronounced as the infections found occurring naturally in the field. Those induced by a puncture at the stem end were deeper than those produced by the other methods.

The failure of the inoculations in the field to develop as severe cases of infection as those occurring in nature may be attributed to the late date on which the inoculations were made and to the very dry condition of the soil. Aside from the depth of the infection at the stem end, the stem-end decays induced by artificial inoculation were very similar in appearance to infections found occurring naturally in commercial fields of Netted Gem potatoes. Wherever the attempt was made, *F. radicola* was recovered from the stem-end infections induced by the inoculations. It is evident, therefore, that *F. radicola* is capable of producing a jelly-end rot of the potato tuber. However, isolations made from such rotted tubers taken from the field have not always revealed *F. radicola* to the exclusion of other fungi. *F. oxysporum* is frequently obtained.

Wollenweber<sup>1</sup> reports the isolation of *F. orthoceras* from jelly-end tubers and thought it the probable cause of the disease. The writer has twice isolated *F. trichothecioides* from such tubers fresh from the field.

Artificial infection of the growing tuber with *F. trichothecioides* under western conditions has never been accomplished. Under conditions of high humidity Jamieson and Wollenweber<sup>2</sup> were able to produce an infection in the growing tuber with this fungus, but their results are not believed to be indicative of what actually takes place in nature in the irrigated West. Tubers infected with jelly-end rot, when kept in a moist chamber for a few days, invariably threw out tufts of *F. radicola* through the lenticels, although from these same tubers with well-advanced stem-end rot other fungi, notably *F. oxysporum*, have been isolated from the interior of the tuber. Carpenter<sup>3</sup> has shown that *F. oxysporum* is capable of producing a similar rot of the potato tuber, and from its frequent occurrence in connection with jelly-end-rot-infected tubers it must be considered as one of the factors involved in producing this type of rot. Other *Fusarium* species, either independently or in conjunction with *F. radicola*, may be in part responsible for the disease.

#### STORAGE EXPERIMENTS

In the fall of 1914 two ordinary 2-bushel sacks filled with Netted Gems infected with jelly-end rot were secured. With a soft blue pencil, a line was drawn around each tuber in such a manner that the blue line separated the decayed from the healthy tissue. The tubers were then sacked and put in storage in the potato cellar of the Jerome Experiment Station, at Jerome, Idaho. Fifty tubers each of Pearls and Idaho Rurals infected with stem-end and lenticel blackrot were secured. On each tuber a blue line was drawn around the stem end at the margin of the infected and healthy tissues. Lenticel infections were marked in the same manner. The marked Pearl and Idaho Rural tubers were then sacked and placed in storage near the similarly treated Netted Gems infected with jelly-end rot.

The storage period was from November 15, 1914, to April 12, 1915. The temperature of the cellar during this period ranged from 32° to 48° F. During the last six weeks of the storage period the minimum temperature was 36°, and for the greater part of this time the temperature approached the maximum of 48°. On April 12 the tubers were removed from the sacks and examined one by one to determine whether the rot had continued to develop. In no case could any perceptible advance in the decay be found. It is apparent that neither jelly-

<sup>1</sup> Wollenweber, H. W. Studies on the *Fusarium* problem. In *Phytopathology*, v. 3, no. 1, p. 24-50. 1 fig., pl. 5. 1913.

<sup>2</sup> Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by *Fusarium trichothecioides*, Wollenw. In *Jour. Washington Acad. Sci.*, v. 2, no. 6, p. 146-152, illus. 1912.

<sup>3</sup> Carpenter, C. W. Op. cit.

end rot nor blackrot makes any progress in storage at a temperature of 48° or under.

This conclusion is further substantiated by results obtained in storing several sacks of blackrot-infected Idaho Rural and Pearl tubers for experimental use in the fall of 1913. Although the infected stock remained in the cellar until the middle of May, 1914, when the cellar temperatures had risen to something over 50° F., the tubers were apparently as sound as at the time they were put in storage. Carpenter<sup>1</sup> has found that when tubers were inoculated with *F. radicola* and kept at a temperature of 12° C. (approximately 53° F.) no rot developed.

#### EFFECT OF PLANTING INFECTED SEED

In the spring of 1915 three plots were planted with infected seed potatoes. Plot 1 was planted with Idaho Rural potatoes every seed piece of which showed infection with *F. radicola*, stem-end blackrot, or lenticel rot. The presence of the fungus was verified by artificial cultures. Plot 2 was planted with Pearl potatoes every seed piece of which was infected with *F. radicola*, stem-end blackrot, or lenticel rot, the presence of the fungus being verified by artificial cultures. Plot 3 was planted with Netted Gem potatoes infected with jelly-end rot. The seed pieces were cut from the stem end, care being exercised to see that at least one healthy eye was present on each seed piece. Cultures from this seed gave a variety of fungi, including *F. radicola* and *F. oxysporum*. Check plots were planted with the same varieties. The seed selected for the check plots was entirely free from disease and was disinfected for 1½ hours in a solution of mercuric chlorid (4 ounces of mercuric chlorid to 30 gallons of water). All of the plots were planted on alfalfa land which had never before been planted to potatoes. The soil was a heavy clay loam of lava-ash formation. Irrigation was given on July 4 and 5, July 16, July 31, and August 1. Throughout the season the plots were kept in a good state of tilth, but they suffered somewhat from lack of moisture during the latter part of August. Table III shows the percentage of disease in the harvested product.

TABLE III.—Percentage of disease in harvested potatoes

Plot No.	Variety.	Condition of seed.	Percentage of disease in tubers.	
			Vascular infection.	Tuber-rots.
1	Idaho Rural.....	Infected with blackrot.....	95	82
2	Pearl.....	do.....	44	40
3	Netted Gem.....	Infected with jelly-end rot.....	16	0
4	Idaho Rural.....	Disease-free, disinfected.....	40	0
5	Pearl.....	do.....	14	1
6	Netted Gem.....	do.....	10	0

<sup>1</sup> Carpenter, C. W. Op. cit.

The vascular infection present in plots 1 and 2 was all of the heavy black type demonstrated to be caused by *F. radicicola*. Numerous cultures from the vascular systems of tubers from these plots gave the fungus. The percentages of rot include all phases of blackrot, including stem-end, lenticel, and eye infections. Strangely enough, no tuber-rots developed in plot 3. Of the tubers from plot 3, 16 per cent showed vascular infection, of which 14 per cent were of the type usually ascribed to *F. oxysporum* and 2 per cent were of the black type caused by *F. radicicola*. Cultures made from the vascular systems of infected tubers in this plot give *F. oxysporum* in all cases of light-brown discoloration and *F. radicicola* in all cases of black vascular discoloration. In the check plots, 1 per cent of blackrot appeared in plot 5. The others were free from all tuber-rots. The vascular infection present in the check plots was for the most part of the type ascribed to *F. oxysporum*. A few tubers showing blackened vascular bundles were found, and *F. radicicola* was isolated from such tissues whenever the attempt was made.

The results clearly show that seed infected with blackrot will produce infection in the resulting product. From the fact that no jelly-end rot resulted from planting jelly-end-infected seed, the conclusion should not be drawn that such seed can not cause infection in the resulting product, but rather that it requires conditions for its development different from those required for the development of blackrot.

#### CONTROL OF BLACKROT

Absolute control of blackrot will be difficult. When potatoes are planted on alfalfa or grain lands blackrot is rarely found if the crop has had sufficient water to make good growth conditions possible. Plantings of disease-free seed potatoes on raw desert lands in 1915 gave as high as 11 per cent of tubers infected with blackrot in the harvested product, whereas plantings of disease-free tubers on alfalfa or grain lands were usually free from the disease, although as high as 5 per cent of infected potatoes were found in the harvested product of one plot on alfalfa land. Judging from the results of three years' observations in commercial fields, it is apparent that losses from blackrot can be reduced to a minimum by planting only on land which has been in cultivation for a number of years and by giving the growing crop the proper amount of water, care, and attention. The crop should be kept in a good growing condition until maturity or frost. Jelly-end rot, on the other hand, has been found in fields where all the conditions of growth were apparently ideal. Some adverse condition, however, is probably responsible for its development. Further research upon jelly-end rot and its cause and occurrence is highly desirable.

Both jelly-end rot and blackrot-infected tubers may be stored with safety, provided the storage cellar is fairly well ventilated and the temperature kept below 50° F.

## SUMMARY

(1) *Fusarium radiculicola* Wollenw. is the cause of a field blackrot of potato tubers in southern Idaho. The disease is confined principally to potatoes of the round type, such as Idaho Rural and Pearl.

(2) *F. radiculicola* is capable of causing a jelly-end rot of potatoes similar to the jelly-end rot of the Burbank group found in southern Idaho, but under actual field conditions other factors are apparently in part responsible.

(3) Neither blackrot nor jelly-end rot makes any progress in storage at or below a temperature of 50° F.

(4) Seed pieces infected with blackrot will bring about infection in the following crop.

(5) *F. radiculicola* is apparently well distributed throughout the desert soils.

(6) Blackrot may be controlled fairly well by planting potatoes only on lands which have been in other crops for a number of years and by providing good conditions for growth.

PLATE XXXIV

Fig. 1, 2, 3, 4.—Types of stem-end blackrot, lenticel rot, and eye rot in Idaho Rural potato tubers. Field material.

Fig. 5, 6.—Longitudinal and cross sections of an Idaho Rural tuber infected with blackrot. Note the blackened vascular system. Field material.

Fieldrot of Potato

PLATE XXXIV



Journal of Agricultural Research

Vol. VII, No. 9

Fieldrot of Potato

PLATE XXXV



Journal of Agricultural Research

Vol. VI, No. 2

PLATE XXXV

Fig. 1.—Netted Gem potato tuber infected with jelly-end rot. A soft bud-end infection may also be seen. Field material.

Fig. 2.—Stem-end view of a Netted Gem tuber infected with jelly-end rot. Field material.

Fig. 3.—Longitudinal section of a Netted Gem tuber infected with jelly-end rot. Field material.

Fig. 4.—Idaho Rural tuber infected with stem-end and lenticel blackrot, after having been kept 10 days in a moist chamber. Tufts of *Fusarium radicola* have appeared. Field material.

Fig. 5.—Idaho Rural tuber infected with lenticel blackrot after having been kept in a moist chamber for 10 days. A single tuft of *F. radicola* has appeared. Field material.

PLATE XXXVI

Fig. 1, 2.—Stem-end blackrot produced by stem-end punctures with a needle carrying *Fusarium radicicola*. Netted Gem and Idaho Rural potato tubers. Laboratory inoculations.

Fig. 3.—Lenticel blackrot produced by spraying the tuber with a spore suspension of *F. radicicola*. Netted Gem tuber. Laboratory inoculation.

Fig. 4.—Same tuber as shown in figure 3; after having been kept a few days longer in the moist chamber. Note the tufts of *F. radicicola* that have appeared.

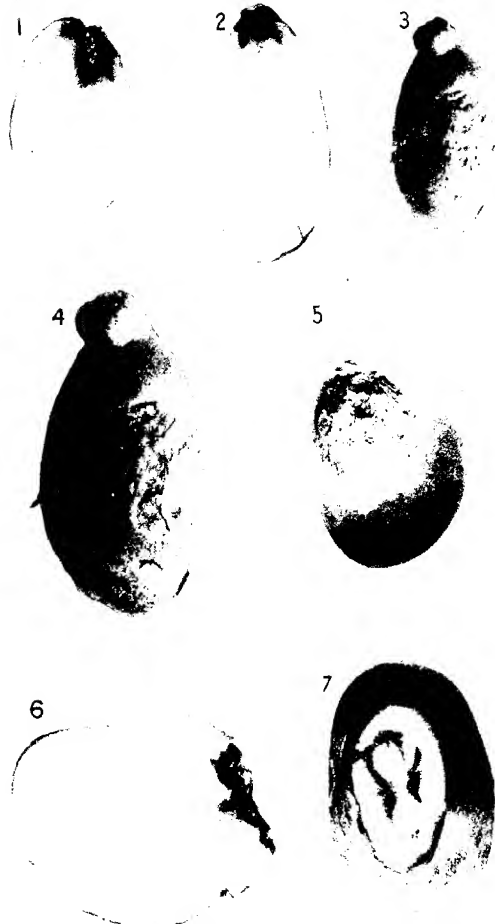
Fig. 5.—Stem-end blackrot produced by spraying an Idaho Rural tuber with a spore suspension of *F. radicicola*. Laboratory inoculation.

Fig. 6.—Stem-end blackrot produced by the inoculation of the tuber stolon. Idaho Rural tuber. Laboratory inoculation.

Fig. 7.—Blackened vascular system produced by the inoculation of the tuber stolon. Idaho Rural tuber. Laboratory inoculation.

Fieldrot of Potato

PLATE XXXVI



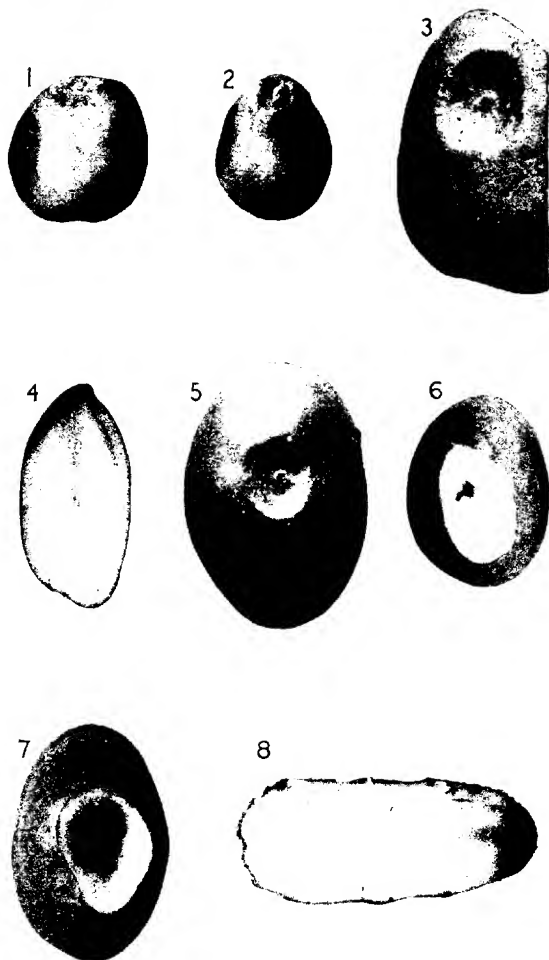


PLATE XXXVII

Fig. 1, 2, 3.—Stem-end and lenticel blackrot produced by spraying the growing tubers with a spore suspension of *Fusarium radicicola*. Idaho Rural potato tubers. Field inoculations.

Fig. 4.—Eye infection produced by spraying the growing tuber with a spore suspension of *F. radicicola*. Netted Gem tuber. Field inoculation.

Fig. 5, 6, 7.—Stem-end blackrot produced by the inoculation of the stolons of growing Idaho Rural tuber. Field inoculation.

Fig. 8.—Stem-end rot of Netted Gem tuber produced by inoculating the stolon of the growing tuber.



# COMPARATIVE STUDY OF THE ROOT SYSTEMS AND LEAF AREAS OF CORN AND THE SORGHUMS

By EDWIN C. MILLER,<sup>1</sup>

Assistant Plant Physiologist, Department of Botany, Kansas Agricultural Experiment  
Station

## INTRODUCTION

During the summers of 1914 and 1915 a series of investigations was conducted to determine the fundamental characteristics possessed by the sorghum plants (*Andropogon sorghum*) which enable them to withstand severe climatic conditions better than the corn plant (*Zea mays*). The results of these investigations will be reported in a series of articles as rapidly as the data are assembled. This paper deals with the comparative study of the root systems and leaf areas of corn, Blackbull kafir, and Dwarf milo. These experiments were carried on at the State Branch Experiment Station at Garden City, Kans. This Station is located in the southwestern part of the State, in latitude 37° 58' north and longitude 100° 55' west (Greenwich), and has an elevation of 2,940 feet.

## EXPERIMENTAL METHODS

### CLIMATIC DATA

The instruments for obtaining the weather data consisted of a thermograph, a hydrograph, a soil thermograph, maximum and minimum thermometers, a psychrometer, a rain gauge, an evaporation tank, and two anemometers. The maximum and minimum thermometers, thermograph, and hydrograph were kept in a standard shelter 4 feet above the ground. One of the anemometers measured the wind velocity at a height of 2 feet and the other at a height of 8 feet. The 2-foot anemometer was connected with a clock attachment so that the wind velocity for each hour was recorded. The bulb of the soil thermograph was buried to a depth of 1 foot.

A portion of the weather records for the growing seasons of 1914 and 1915, grouped in 5-day periods, is given in Table I. This table shows that the climatic conditions of 1914 and 1915 were in marked contrast. The total rainfall for the year 1914 amounted to only 9.7 inches, while that for 1915 totaled 26.77 inches.

<sup>1</sup> Acknowledgments are due Messrs. J. G. Lill and C. B. Brown, of the United States Department of Agriculture, for their aid in obtaining the weather and soil data, and to Mr. M. C. Sewell, formerly superintendent of the Experiment Station at Garden City, Kans., for general assistance in this work.

TABLE I.—Summary of the climatic conditions at Garden City, Kans., for the growing months of 1914 and 1915

Year and month.	Days (in- clusive).	Air temperature (° F.).					Precip- itation.	Evapora- tion.	Wind velocity per hour.
		Average of—			Maxi- mum.	Mini- mum.			
		Mean.	Maxi- mum.	Mini- mum.					
1914.									
May.....	1-5	58	68	47	78	44	<i>Inches.</i> 1.40	<i>Inches.</i> 0.953	<i>Miles.</i> 9.0
Do.....	6-10	65	78	51	92	41	.19	1.484	11.8
Do.....	11-15	53	61	44	72	38	.20	1.135	10.9
Do.....	16-20	62	68	55	79	50	.72	.596	13.6
Do.....	21-25	72	84	59	90	57	.12	1.584	10.2
Do.....	25-31	60	79	57	89	49	1.00	1.294	6.9
June.....	1-5	76	87	65	92	62	.19	1.432	13.0
Do.....	6-10	77	89	64	91	51	.21	1.728	15.2
Do.....	11-15	76	88	63	96	59	.61	1.520	9.3
Do.....	16-20	76	89	62	99	58	.39	1.409	6.4
Do.....	21-25	82	94	69	98	64	.....	1.991	9.9
Do.....	26-30	77	94	59	103	51	.04	1.662	7.7
July.....	1-5	74	85	62	94	53	.15	1.206	6.1
Do.....	6-10	77	91	60	93	53	.10	1.440	4.7
Do.....	11-15	86	99	69	103	64	.....	1.822	5.7
Do.....	16-20	76	87	62	101	58	.21	1.416	7.7
Do.....	21-25	81	94	65	98	64	.10	1.451	5.7
Do.....	26-31	83	98	66	102	64	T.	2.074	5.7
August.....	1-5	77	93	65	95	61	.38	1.477	6.1
Do.....	6-10	77	91	62	95	56	T.	1.792	8.0
Do.....	11-15	77	91	62	95	58	.19	1.474	7.0
Do.....	16-20	82	99	64	102	62	.06	1.959	8.2
Do.....	21-25	77	91	61	99	50	.01	1.745	7.5
Do.....	25-31	73	87	60	94	54	T.	1.563	7.4
September.....	1-5	77	94	60	103	55	.....	1.739	7.5
Do.....	6-10	79	96	64	102	59	.01	1.501	8.6
Do.....	11-15	75	89	58	96	48	.03	1.653	11.4
Do.....	16-20	77	90	60	97	56	.....	1.390	7.6
Do.....	21-25	63	80	44	85	37	.11	1.343	6.4
Do.....	26-30	67	86	51	90	47	.....	1.740	11.1
1915.									
May.....	1-5	53	65	38	76	31	.79	1.187	10.0
Do.....	6-10	56	69	44	81	32	.....	.985	7.7
Do.....	11-15	71	87	55	94	46	.....	1.857	10.8
Do.....	16-20	46	55	39	68	32	2.38	1.324	12.2
Do.....	20-25	67	78	57	90	44	.07	1.069	8.6
Do.....	25-31	55	65	47	72	39	1.15	1.169	8.1
June.....	1-5	65	75	58	81	55	.64	.738	8.7
Do.....	6-10	64	78	52	86	36	.94	1.386	8.6
Do.....	11-15	66	78	53	87	50	.....	1.490	8.0
Do.....	16-20	71	85	61	95	56	.07	1.485	8.8
Do.....	21-25	69	79	58	91	56	.62	1.181	8.5
Do.....	26-30	72	84	59	88	57	.69	1.419	7.1
July.....	1-5	66	77	55	83	49	.57	1.451	8.8
Do.....	6-10	76	90	60	96	54	.51	1.732	8.6
Do.....	11-15	81	97	67	101	64	.06	1.743	6.7
Do.....	16-20	72	84	62	96	56	.15	1.497	7.0
Do.....	21-25	74	85	61	91	56	.13	1.397	5.5
Do.....	25-31	75	74	64	90	62	.24	1.528	6.8
August.....	1-5	69	83	56	90	51	.....	1.012	5.8
Do.....	6-10	70	80	60	94	56	5.11	.860	4.9
Do.....	11-15	72	83	61	86	59	.10	.927	2.7

TABLE I.—Summary of the climatic conditions at Garden City, Kans., for the growing months of 1914 and 1915—Continued

Year and month.	Days (inclusive).	Air temperature (° F.).					Precipitation.	Evaporation.	Wind velocity per hour.
		Average of—			Maximum.	Minimum.			
		Mean.	Maximum.	Minimum.					
1915.									
August.....	16-20	61	80	61	84	57	<i>Inches.</i> 0.03	<i>Inches.</i> 0.790	<i>Miles.</i> 3.2
Do.....	21-25	70	81	60	84	57	.46	1.018	4.4
Do.....	25-31	63	77	50	85	40	.....	1.313	4.7
September.....	1-5	68	83	55	87	51	.82	1.424	7.4
Do.....	6-10	69	81	56	91	54	.....	1.020	6.3
Do.....	11-15	71	84	60	97	53	T.	.983	7.2
Do.....	16-20	60	82	55	87	39	.20	1.072	5.2
Do.....	21-25	66	76	58	84	50	1.00	.804	18.2
Do.....	25-30	56	67	48	78	44	.25	.665	4.4

During the growing months of May, June, July, August, and September in 1914 the rainfall amounted to only 6.42 inches, while during the same months in 1915 it amounted to 17.88 inches. Table II gives the number of inches of rainfall for each month during 1914 and 1915.

TABLE II.—Rainfall (in inches) at Garden City, Kans., in 1914 and 1915

Month.	Year.		Month.	Year.	
	1914	1915		1914	1915
January.....	None.	None.	July.....	0.56	1.66
February.....	Trace.	2.53	August.....	.64	6.40
March.....	Trace.	.18	September.....	.15	2.27
April.....	1.74	2.67	October.....	1.48	1.79
May.....	3.63	4.59	November.....	Trace.	.12
June.....	1.44	2.56	December.....	.06	1.6

The summer of 1914 was much warmer than that of 1915, and the evaporation for each of the five growing months, with but one exception, was appreciably lower in the latter year than in the former. The evaporation from a free water surface for each month of the growing season is given in Table III.

TABLE III.—Evaporation (in inches) at Garden City, Kans., for the growing months of 1914 and 1915

Month.	Year.		Month.	Year.	
	1914	1915		1914	1915
May.....	7.046	7.593	August.....	10.050	5.020
June.....	9.942	7.690	September.....	9.366	6.037
July.....	9.403	9.258			

The evaporation during 5-day periods for the two growing seasons is shown graphically in figure 1.

#### GENERAL OUTLINE OF THE WORK

The experiments herein reported were conducted with Pride of Saline corn, Blackhull kafir, and Dwarf milo. The plants were grown both in the field and in large galvanized-iron cans. The investigations with the plants in the field included (1) the isolation of the root systems of

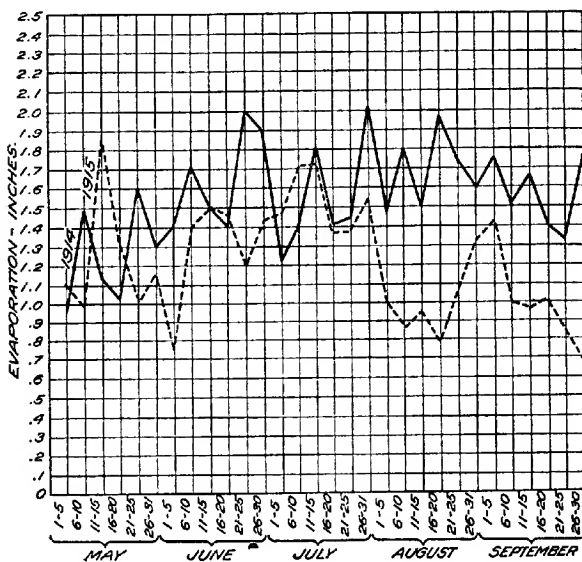


FIG. 1.—Evaporation from a free water surface (tank) at Garden City, Kans., during the growing seasons of 1914 and 1915.

corn, Blackhull kafir, and Dwarf milo at three stages of their growth; (2) a study of these root systems in relation to their general extent, as well as the number of their primary and secondary roots; (3) a comparative study of the leaf and sheath areas of these three plants at four periods of their growth; (4) a study of the soil-moisture content and the depth of root penetration.

The plants grown in the large iron containers furnished the material for a study of the relative dry weights of the roots and aerial portions of corn, Blackhull kafir, and Dwarf milo.

## CULTURAL METHODS

The soil in which the plants were grown is known as a sandy loam of the Richfield series and shows very little difference in its texture in the upper 10 feet. Tables VIII and IX give the moisture equivalent and the wilting coefficient (1, p. 56-73)<sup>1</sup> for the soil at each foot to a depth of 10 feet on the plots which were used in 1914 and 1915, respectively.

The plants were grown on plots which had been in Dwarf milo the previous season. The land was plowed in the fall to a depth of 6 inches and then irrigated with approximately 8 to 10 inches of water or until the soil was saturated to a depth of from 3 to 4 feet. It received no further attention until spring, when it received several shallow cultivations, was then harrowed, and before planting was leveled with a float.

In order that the plants might be under the same conditions, the corn, kafir, and milo were planted in alternate rows on the same plots. On May 23, 1914, and on May 26, 1915, the crops were surface-planted in rows 44 inches apart. After the plants were a few inches in height the corn in the rows was thinned to a distance of  $1\frac{1}{4}$  to 2 feet between the plants, Blackhull kafir from 1 to  $1\frac{1}{2}$  feet, and the Dwarf milo from 8 inches to 1 foot. The plants were kept free from suckers at all times during the growing season. The plots were scraped with a hoe as often as was necessary to keep them free from weeds, but no other cultivation was given. After the fall irrigation the plots received no water other than that from the rainfall.

The relative weights of the root systems and aerial portions of the corn, Blackhull kafir, and Dwarf milo were obtained from plants grown in large sealed metal containers. These cans were made of 22-gauge galvanized iron and were 24 inches in height with a diameter of about 15 inches; and in this experiment each can contained from 100 to 110 kgm. of soil. The surface foot of the field soil was worked through a  $\frac{1}{4}$ -inch mesh screen and then thoroughly tamped in the cans. This soil was in good tilth, and for both seasons had a moisture content of 20 to 21 per cent (dry basis). This moisture content was kept approximately constant during the growing season by weighing the cans every 48 hours and then replacing the water that had been lost by the method used by Briggs and Shantz (2) in their work on the water requirement of plants. Different numbers of plants were grown in each can, as will be shown in the tables that record the data for this part of the work.

## ISOLATION OF ROOT SYSTEMS IN THE FIELD

The root systems of plants growing under field conditions were isolated by a modification of the method devised by King (5).<sup>2</sup> This method, stated briefly, consists of the isolation of a prism of soil con-

<sup>1</sup> Reference is made by number to "Literature cited," p. 321.

<sup>2</sup> The work of other investigators concerning the development of root systems will be mentioned in this article only in so far as it is necessary to give a clear discussion of the experiments reported. The studies that have been made by other investigators on the development of the root systems of agricultural plants have been reviewed in detail elsewhere by the writer.

taining the plants whose root systems are desired and then placing over this block of earth a wire cage of such a shape and size as to fit closely to the vertical sides of the block. Numerous small wires are then run through the prism of earth and fastened to each side of the cage. The plants are fastened to the cage at the surface of the soil and the roots washed free from dirt by means of a stream of water. When the earth is washed away, the main roots remain suspended on the cross wires in the same position that they occupied in the soil.

This method is open to criticism, first; because in order to use it with any degree of satisfaction the prism of soil must be limited to about 18 inches in thickness, and on this account one obtains only a section of the root system. Furthermore, the main roots of the plant may not be in the prism of soil which has been isolated; therefore, when the soil is washed away, only a poor representation of the root system is obtained. Finally, although the primary roots of the plant remain on the wires in the same position that they occupied in the soil, it is impossible to retain all the finer roots in their normal position. No method has been devised, so far as is known to the writer, whereby the root systems of mature plants growing in the field under natural conditions can be isolated intact. The method of Rotmistrov (6) for obtaining complete root systems is open to criticism because root systems certainly would not develop normally in so small a volume of soil. For a comparative study of the general nature of the root systems of plants, growing under field conditions, the modified method of King as used in these experiments seems to be the least objectionable.

In the work reported in this paper, sections of the root systems were obtained crosswise of the rows. The prisms of soil varied from 15 to 18 inches in thickness and were isolated by digging a trench 2½ feet wide around them. After the isolation of a prism of soil, a wooden framework of light material was fitted snugly over it. Ordinary wire fencing with a 4- to 6-inch rectangular mesh was placed on two sides of the framework (Pl. XXXVIII, fig. 1, 2). This was found to be much more satisfactory than the poultry netting used by King and Ten Eyck, since the small mesh of such netting made it impossible to photograph the root systems with any degree of satisfaction after they had been isolated. The plant stubs and root crowns were held in place by wiring them to narrow strips of inch boards which were placed crosswise of the soil block at the surface of the soil and nailed to both sides of the framework of the cage. This method is much more convenient and simple than the one used by King (5) and Ten Eyck (9, 10, 11). In order to hold the plants in place, these investigators removed the upper portion of the soil surrounding the crown of the plant, and replaced it by a plaster of Paris cast.

For cross wires, ordinary broom wire was found to be the most satisfactory. Owing to the compactness of the soil, a ¼-inch iron rod pointed

at one end and provided with a wooden handle at the other was employed to make a passage through the soil block for the cross wires; (Pl. XXXVIII, fig. 2). In the upper 2 feet of soil the cross wires were pushed through the block of soil at intervals of 3 to 4 inches on both the vertical and horizontal wires of the cage, while below that depth they were placed at the intersections only of the vertical and horizontal wires. In the isolation of the root systems of two mature plants, between 200 and 250 cross wires were pushed through the soil prism.

Several methods of washing the soil away from the roots were tried, but the following was found the most desirable: The trench around the block of soil was partially filled with water from an irrigation ditch near by; and then by means of a pitcher pump connected with a  $\frac{3}{4}$ -inch pipe of convenient length the water was pumped into a piece of galvanized-iron eaves trough and allowed to flow gently on the prism of soil (Pl. XXXVIII, fig. 3). In this manner the same water could be used over and over again. As soon as any of the larger roots were exposed they were carefully tied to the cross wires so that they would not be moved from their original position by the further washing. When the dirt that had been washed from the soil prism had filled the trenches to the surface of the water, the washing was discontinued and the water allowed to soak away. The soil that had been washed into the trenches was then removed, the trench again partially filled with water, and the washing continued. This routine, especially in working with mature plants, had to be repeated several times. After the soil had been washed from all the roots, the cages containing them were taken up, the unused cross wires removed and the root systems studied and photographed.

#### ISOLATION OF THE ROOT SYSTEMS FROM LARGE VESSELS

The following method was used in the isolation of the root systems of the plants that were grown in large galvanized-iron cans:

As soon as the aerial portions of the plants were harvested, the soil contained in the can was emptied upon a cleared space; and all the larger roots were removed from the soil by carefully working it over, a handful at a time. In order to separate the soil from the root particles still remaining in it, as much of the soil as possible was shaken through a sieve with a  $\frac{1}{16}$ -inch mesh. In this manner all the finer root portions, together with the larger soil particles, remained upon the screen. The root remnants and the soil particles on the sieve were then placed in a vessel and covered with a large excess of water, which was stirred vigorously until all the lumps of soil had disintegrated. All the root remnants floated to the surface of the water, and as soon as the soil in the vessel had settled, they were removed by pouring the water upon the fine sieve. All the roots which were obtained from each can were placed upon the fine screen and washed carefully a number of times until, so

far as could be seen, they were free from sand particles. The roots were then dried in a hot-air oven at 105° C. and their dry weight obtained.

#### DETERMINATION OF THE LEAF AREA

For obtaining the leaf and sheath areas five representative plants of the corn, kafir, and milo, respectively, were selected at the desired stage of growth. Their leaves and sheaths were cut into convenient pieces, and the outlines of these portions were carefully traced with a hard lead pencil on ordinary unruled paper. The outlines thus obtained were traced with a polar planimeter and the inclosed area calculated. In dealing with that portion of the leaf which was not yet fully unfolded, care was taken that the measurements included only that surface of the unfolding leaf that was exposed to the air.

#### GENERAL DISCUSSION OF EXPERIMENTAL DATA

##### EXTENT OF THE ROOT SYSTEMS

The root systems of corn, kafir, and milo growing in the field were isolated at four stages of growth in 1914 and at three stages in 1915. A summary of the general extent of the root systems of these plants is given in Table IV.

TABLE IV.—General summary of the root systems isolated during the summers of 1914 and 1915 at Garden City, Kans.

Date.	Crop.	Height of plants.	Greatest depth of root penetration.	Greatest lateral extent of roots.	Greatest length of a single root.	General remarks.
		<i>Ft. in.</i>	<i>Ft. in.</i>	<i>Ft. in.</i>	<i>Ft. in.</i>	
1914.						
June 24	Corn, Pride of Saline....	1 6	1 4	2 9	3 3	4 fully unfolded and 4 partially unfolded leaves.
	Kafir, Blackhull.....	1 0	1 6	3 0	3 5	Do.
	Milo, Dwarf.....	1 0	1 6	3 0	3 7	Do.
July 17	Corn, Pride of Saline....	3 6	3 0	3 6	3 8	8 fully and 6 partially unfolded leaves.
	Kafir, Blackhull.....	3 6	2 6	4 0	5 0	6 fully and 4 partially unfolded leaves.
	Milo, Dwarf.....	2 6	2 9	3 0	4 2	"Rooting."
Aug. 1	Corn, Pride of Saline....	5 6	4 0	2 6	4 6	"Shooting."
	Kafir, Blackhull.....	4 0	4 0	3 6	5 8	Heading.
	Milo, Dwarf.....	3 0	4 0	3 6	5 0	Seed in milk stage.
Aug. 25	Corn, Pride of Saline....	6 0	6 0	3 0	7 0	End of vegetative growth, grains glazed.
	Kafir, Blackhull.....	5 0	6 0	3 10	8 2	Seed in milk.
	Milo, Dwarf.....	3 0	6 0	3 6	7 6	Seed fully ripe.
1915.						
July 10	Corn, Pride of Saline....	2 6	2 3	2 10	3 0	4 fully and 4 partially unfolded leaves.
	Kafir, Blackhull.....	1 0	1 6	2 0	2 3	Do.
	Milo, Dwarf.....	1 0	2 0	2 0	2 6	Do.
30	Corn, Pride of Saline....	5 0	4 6	3 8	6 0	Tassel peeping.
	Kafir, Blackhull.....	3 6	4 6	3 8	6 3	7 fully unfolded and 6 partially unfolded leaves.
	Milo, Dwarf.....	3 0	4 6	3 8	6 0	Blooming.
Sept. 3	Corn, Pride of Saline....	7 0	6 0	3 8	7 0	Early milk stage.
	Kafir, Blackhull.....	6 0	6 0	3 8	8 8	Blooming.
	Milo, Dwarf.....	3 6	6 0	3 8	6 8	Seed in milk stage.

**Stage I.**—At this period of growth, the plants of Dwarf milo and Blackhull kafir had reached a height of 1 foot and had four fully and four partially unfolded leaves, while the corn plants with the same number of leaves had a height of 1 foot 6 inches. In 1914 the plants reached this stage on June 24, four weeks from the time of planting the seed; but in 1915, owing to cool weather, they did not reach this stage until July 10, six weeks after seeding (Pl. XLIII, fig. 1).

In 1914 the greatest depth reached by the root system of the corn plant at this stage was 1 foot 4 inches, while the greatest depth of the kafir and milo roots was 1 foot 6 inches. At this time the roots of the corn extended horizontally to a distance of 2 feet 9 inches, while in the same direction the roots of both kafir and milo extended 3 feet (Pl. XXXIX, fig. 3). The depth of root penetration for corn and kafir at this stage was practically the same in 1915 as in 1914, but Dwarf milo exceeded the depth reached the previous year by 6 inches. The maximum lateral extent of the corn roots was the same as in 1914, but it was 1 foot less for the kafir and milo (Pl. XXXIX, fig. 2, 4).

At this time the differences exhibited by these three plants in their method of rooting were very slight. The number of primary roots which penetrated to a depth of a foot was between 12 and 15 for each plant, but more of the first primary roots of the corn took a horizontal direction than did those of the kafir and milo. On this account more of the primary roots of the latter penetrated to the maximum depth than did those of the corn plant. The secondary roots of all the plants grew both upward and downward from the primary roots, so that at this stage the upper foot of soil was filled with a network of roots to within  $\frac{1}{4}$  inch of the surface.

**Stage II.**—The root systems at this period of growth were isolated only in 1914. At this time the corn plants had reached a height of  $3\frac{1}{2}$  feet and had 8 fully and 6 partially unfolded leaves, while Blackhull kafir, with approximately the same number of leaves, had a height of  $2\frac{1}{2}$  feet. The Dwarf milo plants had from 9 to 10 fully unfolded leaves, including the "boot" leaf, and stood  $2\frac{1}{2}$  feet high. The plants reached this stage on July 17, seven weeks from the time of planting (Pl. XLIV, fig. 1).

The greatest depth reached by the corn roots at this time was 3 feet, while the maximum depth for Blackhull kafir and Dwarf milo was 2 feet 6 inches and 2 feet 9 inches, respectively. The greatest lateral extent reached by the roots of corn and Dwarf milo at this period was 3 feet, while the roots of standard kafir extended horizontally for a distance of 4 feet. The tendency of the first primary roots of the corn to take a more horizontal direction than those of the sorghums is well shown at this stage (Pl. XXXIX, fig. 1).

It was found that the later roots of the corn take the same general direction as do those of Blackhull kafir and Dwarf milo, and that the maximum depth of root penetration is practically the same for all three plants.

Stage III.—In 1914 the roots of the three plants were isolated about the first of August, 10 weeks from the time of planting. The corn at this stage was shooting and had a height of  $5\frac{1}{2}$  feet, while Blackhull kafir was heading and stood 4 feet high. The seed of the Dwarf milo was in the milk stage, and the plant had reached a height of 3 feet.

The greatest depth of root penetration at this stage was 4 feet for all the plants. The maximum lateral extent of the roots of corn was  $2\frac{1}{4}$  feet, while the roots of both Blackhull kafir and Dwarf milo showed a maximum horizontal extent of  $3\frac{1}{2}$  feet (Pl. XI, fig. 2).

The roots at this stage were isolated on July 17, 1915, when the plants had reached the same age at which they were examined the previous year. The corn at this date stood 5 feet high, and the tassel was just beginning to show. Blackhull kafir stood  $3\frac{1}{2}$  feet high and had seven fully and five partially unfolded leaves. The Dwarf milo was blooming and had a height of 3 feet.

The maximum depth and lateral extent of the roots at this stage was found to be approximately the same for all three plants. The greatest depth of the roots was  $4\frac{1}{2}$  feet, while the greatest extent in a horizontal direction was approximately  $3\frac{2}{3}$  feet.

Stage IV.—The root systems at this stage were isolated on August 25, 1914, when the plants were 13 weeks old. The corn had reached a height of 6 feet and the grain was in a glazed condition. The seed of Blackhull kafir was in the milk stage and the plants which stood 5 feet high had reached their maximum vegetative growth. The seed of the Dwarf milo was fully ripe, and the plants had made little if any growth since the previous stage (Pl. XLIV, fig. 2).

The roots of all three plants were found to reach a maximum depth of 6 feet, while the greatest lateral extent for all three was between 3 and 4 feet (Pl. XL, fig. 1).

In 1915 the plants had not reached their full vegetative growth until September 3, and even at that date they were not nearly as mature as those examined at the same age in 1914. The corn was 7 feet high, and the grain was in the early milk stage. Blackhull kafir was in bloom and had a height of 6 feet, while the grain of the Dwarf milo was in the milk stage and the plants stood  $3\frac{1}{2}$  feet high.

The maximum depth of the root systems was 6 feet for each plant, while while the maximum extent horizontally for each was  $3\frac{2}{3}$  feet (Pl. XLI, fig. 1, 2).

Both the primary and secondary roots of Dwarf milo and Blackhull kafir at all stages of growth were more fibrous than those of the corn. The length of the secondary roots was found to be approximately the same for the three plants at any given stage of growth. The secondary roots of kafir and Dwarf milo broke so easily in the washing process that it was almost impossible to obtain them intact from the soil which was used in this experiment (Pl. XLII, fig. 1, 2).

## NUMBER OF SECONDARY ROOTS

It has been shown in the foregoing discussion of the isolation of the root systems of corn, Blackhull kafir, and Dwarf milo at the various periods of growth, that no marked differences were to be observed between these plants in regard to the number and general extent of their primary roots. It was thought advisable on this account to make a study of the number of secondary roots possessed by the three plants at different stages of growth.

After the isolated root systems had been studied and photographed the primary roots of each plant were cut into inch lengths and the number of the secondary roots originating from each unit of length was determined under a dissecting microscope. The results of this investigation for all the stages of root growth examined in 1914 and 1915 are shown in Table V. It was found from 321 observations of the roots of the corn, 311 of Dwarf milo and 210 of Blackhull kafir that the number of secondary roots per unit of length of primary root was approximately twice as great for the two sorghums as for the corn. This fact stands out strikingly not only for each year but for all the different stages of the development of the root systems (Pl. XLII, fig. 1, 2).

TABLE V.—Number of secondary roots per unit of length of primary roots of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.

Year and crop.	Stage of growth (height of plants in feet).	Number of observations.	Average number of roots per inch.	Average number of roots per centimeter.
1914.				
Corn, Pride of Saline.....	1½	33	15	6
	3½	37	17	7
	6	37	12	5
	6	32	11	4
Milo, Dwarf.....	1	21	25	10
	2½	54	29	12
	3	72	26	10
Kafir, Blackhull.....	2½	40	31	13
	5	60	26	10
1915.				
Corn, Pride of Saline.....	1½	50	16	6
	5	65	12	5
	7	47	12	5
	7	24	13	9
Milo, Dwarf.....	3	79	30	12
	3½	70	25	10
	5	40	20	8
Kafir, Blackhull.....	5	70	20	8
	6	70	20	8

## WEIGHT OF THE ROOTS AND AERIAL PORTIONS OF THE PLANTS

A comparative study was made of the dry weight of the aerial parts and roots of corn, Blackhull kafir, and Dwarf milo in 1914, and for these three plants and Dwarf Blackhull kafir in 1915. The root systems that

were isolated for this study were obtained from mature plants which were grown primarily for transpiration studies in the large metal cans previously described. The plants made a vigorous growth and compared very favorably in every way with the plants that were grown under field conditions.

Three corn plants were grown in each can during both seasons. In 1914 the corn reached a height of 5 feet, and in 1915 it stood 6 feet high, but no grain was produced in either season. In 1914 six Dwarf milo plants were grown in each can, but in 1915 the number of plants was reduced to three to each can. Six Blackhull kafir plants were grown to each can in 1914 and three plants to each can in 1915.

The Dwarf milo reached a height of 3 feet in 1914, while in 1915 it stood 4½ feet high. The Blackhull kafir plants attained a height of 5 feet in 1914, but in 1915 they reached a height of 6 feet. Dwarf Blackhull kafir was planted during the season of 1915 only, and three plants were grown in each can. These plants reached a height of 4½ feet. The results for the two seasons are shown in Table VI.

TABLE VI.—Relative weight of the roots and aerial portions of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.

1914						
Crop and can No.	Number of plants.	Weight of stem, leaves, and grain.	Weight of stem and leaves.	Weight of roots.	Ratio of the weight of stem, leaves, and grain to weight of the roots.	Ratio of the weight of the stem and leaves to the weight of the roots.
Milo, Dwarf:		Gm.	Gm.	Gm.		
1.....	6	187.3	115.5	11.7	16	9.8
2.....	6	161.5	121.1	10.7	15	11.3
3.....	6	173.9	128.7	12.9	13.4	9.9
4.....	6	184.4	105.1	12.0	15.3	8.7
5.....	6	161.7	102.9	12.0	13.4	8.5
6.....	6	150.7	91.2	9.5	16.8	9.6
Average ratio.....					15.0	9.6
Kafir, Blackhull:						
7.....	4	217.9	163.4	16.5	13.2	9.9
8.....	5	234.1	167.4	12.9	18.1	12.9
9.....	5	212.6	157.1	14.2	14.9	11.0
10.....	6	219.5	159.0	13.8	15.9	11.5
11.....	4	175.6	123.6	10.9	16.1	11.3
12.....	6	257.3	180.0	20.8	12.3	8.8
Average ratio.....					15.0	10.9
Corn, Pride of Saline:						
13.....	3		150.6	13.7		10.7
14.....	3		153.9	15.9		9.6
15.....	3		131.4	15.6		8.4
16.....	3		163.7	16.4		9.9
Average ratio.....						9.6

TABLE VI.—Relative weight of the roots and aerial portions of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.—Continued

1915

Crop and can No.	Number of plants.	Weight of stem, leaves, and grain.	Weight of stem and leaves.	Weight of roots.	Ratio of the weight of stem, leaves, and grain to weight of the roots.	Ratio of the weight of the stem and leaves to the weight of the roots.
<b>Milo, Dwarf:</b>						
		Gm.	Gm.	Gm.		
2.....	3	214.6	111.5	13.5	15.8	8.2
3.....	3	226.4	111.8	12.7	17.8	8.8
5.....	3	231.4	125.8	14.0	16.5	8.9
6.....	3	223.3	121.3	22.4	<sup>a</sup> (9.9)	<sup>a</sup> (5.4)
7.....	3	233.3	123.7	15.0	15.5	8.2
8.....	3	217.6	110.0	14.0	15.5	7.8
11.....	3	230.5	115.8	16.8	13.7	6.8
12.....	3	225.8	117.5	15.0	15.0	7.8
Average ratio.....					15.7	8.0
<b>Kafir, Dwarf Black-hull:</b>						
13.....	3	249.7	142.7	16.0	15.6	8.9
14.....	3	221.8	133.4	13.6	16.3	9.8
15.....	3	257.8	137.9	15.4	16.7	8.9
16.....	2	168.8	97.1	10.4	16.2	9.3
17.....	3	230.2	135.1	16.9	13.6	8.0
Average ratio.....					15.7	8.9
<b>Kafir, Blackhull:</b>						
18.....	3	341.7	215.0	19.0	17.9	11.3
21.....	2	219.3	147.2	14.6	15.0	10.0
53.....	3	209.7	207.3	25.0	11.9	8.2
54.....	3	287	206.3	23.5	12.2	8.7
55.....	3	310.3	213.1	14.7	<sup>a</sup> (21.1)	<sup>a</sup> (14.4)
56.....	3	342.8	253.2	21.0	16.3	12.0
57.....	3	333.8	219.5	20.1	16.6	10.9
58.....	3	354.2	244.6	14.7	<sup>a</sup> (24.0)	<sup>a</sup> (16.6)
Average ratio.....					14.9	10.1
<b>Corn, Pride of Sa-line:</b>						
24.....	3		205.6	30.5		6.7
25.....	3		252.5	33.1		7.6
26.....	3		234.4	26.9		9.0
27.....	3		202.4	28.2		7.1
28.....	3		211.2	33.1		6.3
29.....	3		228.3	31.7		7.2
42.....	3		239.7	24.7		9.7
43.....	3		249.3	28.1		8.8
Average ratio.....						7.8

<sup>a</sup> Not included in the average.

The root systems of Dwarf milo and Blackhull kafir were isolated from six cans in 1914 and from eight cans in 1915. The average ratio of the dry weight of the grain and of the stem and leaves of Dwarf milo to the dry weight of the roots was as 15 to 1 in 1914 and as 15.7 to 1 in 1915.

The dry weight of the stem and leaves was 9.6 times the weight of the roots in 1914, and 8 times their weight in 1915. In 1914 the dry weight of the grain, stem, and leaves of Blackhull kafir was 15 times that of the roots, while the ratio of the dry weight of the stem and leaves to the dry weight of the roots was as 10.9 to 1. The average ratio of the weight of all the aerial parts to the root weight in 1915 was as 14.9 to 1, while the weight of the stem and leaves was 10.1 times that of the roots. In 1914 root systems of corn were obtained from 4 cans and from 10 cans in 1915. The average ratio of the weight of the stem and leaves to the weight of the roots was 9.6 in 1914 and 7.8 in 1915. The roots of Dwarf Blackhull kafir were isolated from five cans in 1915. The weight of all the aerial parts was 15.7 times that of the roots, while the ratio of the weight of the stem and leaves to the weight of the roots was 8.9 to 1.

For the purpose of comparison the results obtained by various investigators for the relative weights of the tops and roots of plants are given here. It must be borne in mind, however, that the relative weights of the roots and aerial portions of plants vary according to the conditions under which they are grown. It has been shown (4, 8, 12) that, among other factors, the soil-moisture content and the amount of available plant nutrients are important in determining the ratio of the weight of the tops of plants to their root weight. Hellriegel (3) found the ratio of the aerial portions of mature barley and oat plants to the weight of their roots to be 11.6 to 1, and 6.6 to 1, respectively. Schulze (7) reports the ratio of the weight of the aerial portions to the weight of the roots to be 10.8, 13.5, and 11.1, respectively, for mature wheat, barley, and oat plants. King (5) found the weight of the aerial part of mature corn to be 7 times that of the root weight, while Kiesselbach (4) found the ratio of the weight of the tops to the root weight to be 8.5 for corn plants grown in a soil with a water content of 98 per cent and 5.2 for plants growing in a soil with a water content of 20 per cent.

#### SOIL-MOISTURE CONTENT AND THE DEPTH OF ROOT PENETRATION

In order to be able more exactly to define the conditions under which the plants used for root examinations were grown, soil samples for moisture determinations were taken at intervals of from 10 to 14 days from the plots upon which the corn, standard kafir, and Dwarf milo grew. Since the moisture content of the soil was determined a few days before or a few days after the isolation of the various root systems, it was possible to compare the depth of the penetration of the roots with the depth of the moisture depletion of the soil.

The results of these observations are given in Table VII. The moisture content of the soil for each foot to a depth of 10 feet is shown for several periods of the two growing seasons. The depth of the root penetration was determined from the root systems isolated at the various stages

which have already been described. The moisture equivalent, together with the wilting coefficient obtained from it by the formula of Briggs and Shantz (1, p. 56-73) for each foot of soil, is also included therein.

TABLE VII.—*Soil-moisture content and depth of root penetration of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.*

Date.	Percentage of moisture at a depth of —										Greatest depth of roots.		
	1 foot.	2 feet.	3 feet.	4 feet.	5 feet.	6 feet.	7 feet.	8 feet.	9 feet.	10 feet.	Corn.	Kafir.	Milo.
<b>1914.</b>													
June 5.....	22.9	22.5	21.1	22.7	23.5	21.2	16.1	17.0	16.4	14.5	Feet.	Feet.	Feet.
July 2.....	14.6	20.2	21.2	22.8	22.1	21.8	.....	.....	.....	.....	1	1½	1½
12.....	11.8	17.5	19.5	22.6	24.0	21.4	.....	.....	.....	.....	.....	.....	.....
21.....	10.6	13.3	14.5	19.4	22.8	21.7	.....	.....	.....	.....	3	2½	3
29.....	8.7	13.1	13.5	15.8	21.4	21.0	.....	.....	.....	.....	4	4	4
Aug. 9.....	9.4	13.5	13.3	14.5	20.3	19.5	.....	.....	.....	.....	6	6	6
22.....	8.4	13.4	12.4	14.4	19.9	19.7	.....	.....	.....	.....	6	6	6
Sept. 6.....	7.7	12.2	12.4	12.9	15.6	19.1	18.2	18.9	16.7	13.0	6	6	6
Wilting coefficient of Briggs and Shantz.....	12.7	14.5	14.5	16.3	17.1	16.1	15.7	15.7	15.8	15.0	.....	.....	.....
Moisture equivalent.....	13.4	16.7	16.7	18.0	17.5	16.6	16.0	15.9	16.2	17.6	.....	.....	.....
<b>1915.</b>													
June 18.....	21.0	21.4	21.7	18.5	15.5	16.0	16.6	19.1	18.8	19.2	.....	.....	.....
29.....	20.3	20.8	20.8	17.9	16.8	16.7	17.1	19.3	18.4	19.7	.....	.....	.....
July 12.....	16.2	20.8	20.2	17.8	19.0	15.5	.....	.....	.....	.....	1¾	1½	2
24.....	12.9	15.7	17.8	17.4	17.4	16.5	.....	.....	.....	.....	.....	.....	.....
Aug. 6.....	14.2	15.2	16.4	16.0	16.1	16.4	17.9	16.6	20.6	20.2	4½	4½	4½
16.....	21.0	20.7	17.7	17.2	16.4	15.5	16.0	19.0	19.5	20.4	.....	.....	.....
25.....	17.9	17.9	15.9	15.5	15.6	16.2	19.8	19.9	20.7	20.9	6	6	6
Wilting coefficient of Briggs and Shantz.....	13.3	14.1	14.9	13.6	13.4	11.9	12.4	12.1	11.7	13.0	.....	.....	.....
Moisture equivalent.....	24.4	25.9	27.5	25.1	24.6	21.9	22.8	22.3	20.5	21.9	.....	.....	.....

The season of 1914 was especially favorable for such an observation, since the rainfall for the last half of June amounted to only 0.44 inch, and for July and August 0.56 and 0.64 inch, respectively. This amount of rainfall, a little over 1½ inches for the 2½ months, came at 12 different periods, so that with the exception of the first foot of soil no influence was exerted by the rainfall upon the original soil-moisture content. The season of 1915 was not so favorable for an observation of this kind, but the results, while not so striking as those of 1914, show the same facts. It should be borne in mind in studying Table VII that in 1914 the soil samples which were taken on July 2 and 21 were procured from five to six days after the isolation of the root systems whose depths are recorded for that date. Furthermore, in 1915 the samples for July 12 and August 6 were taken two and six days, respectively, after the recorded depths of the root systems.

The results of these experiments for both seasons seem to show that there was little if any depletion of the soil moisture below the depth to which the roots penetrated.

LEAF AND SHEATH AREAS<sup>1</sup>

The leaf and sheath areas of corn, Blackhull kafir, and Dwarf milo were determined at four stages of growth in 1914. The results of these measurements are shown in Table VIII. Figures 2 and 3 represent these areas graphically.

TABLE VIII.—Dry weight, leaf and sheath areas of corn, kafir, and milo at different stages of growth in 1914 at Garden City, Kans.

Plant and period of growth.	Plant No.	Dry weight of leaves and stems.	Leaf area.		Sheath area.		
			Gm.	Sq. in.	Sq. cm.	Sq. in.	Sq. cm.
STAGE I.							
Corn, Pride of Saline, June 24, 1914, one month from time of planting.	1	12.3	272.2	3,750.1	24.1	155.4	155.4
	2	11.4	230.0	1,497.5	15.0	96.7	96.7
	3	15.4	205.7	1,542.6	23.9	154.4	154.4
	4	9.6	205.3	1,327.6	12.8	82.6	82.6
	5	9.2	210.2	1,355.7	14.5	93.9	93.9
Average.....		11.5	241.0	1,553.9	18.0	116.6	116.6
Kafir, Blackhull, June 24, 1914, one month from time of planting.	1	9.0	141.3	911.7	15.9	102.8	102.8
	2	11.4	191.9	1,238.1	15.7	100.9	100.9
	3	7.5	145.0	935.2	10.2	65.8	65.8
	4	7.5	133.0	890.1	9.8	63.2	63.2
	5	5.4	116.8	753.4	8.4	54.2	54.2
Average.....		8.1	146.7	945.7	12.0	77.3	77.3
Milo, Dwarf, June 24, 1914, one month from time of planting.	1	6.1	140.0	903.2	9.6	62.2	62.2
	2	6.6	138.3	892.0	10.5	67.8	67.8
	3	7.0	149.0	961.1	9.7	62.6	62.6
	4	7.3	155.6	1,003.8	10.4	66.8	66.8
	5	5.5	122.1	787.3	9.1	58.8	58.8
Average.....		6.5	141.0	909.5	9.8	63.6	63.6
STAGE II.							
Corn, Pride of Saline, July 7, 1914, six weeks from time of planting.	1	51.1	902.7	5,822.5	71.2	459.0	459.0
	2	49.7	842.3	5,433.1	67.8	437.3	437.3
	3	49.5	848.6	5,344.2	81.2	523.5	523.5
	4	50.0	877.3	5,638.6	54.0	348.3	348.3
	5	54.7	754.3	4,865.5	67.3	434.2	434.2
Average.....		51.0	841.0	5,424.7	68.3	440.4	440.4
Kafir, Blackhull, July 7, 1914, six weeks from time of planting.	1	27.8	372.3	2,401.6	26.3	169.6	169.6
	2	31.4	438.0	2,821.4	35.8	231.2	231.2
	3	31.0	454.0	3,122.1	42.7	275.7	275.7
	4	29.9	420.7	2,713.8	41.0	264.4	264.4
	5	27.5	338.2	2,181.4	29.3	189.3	189.3
Average.....		29.5	406.6	2,835.8	35.0	226.0	226.0
Milo, Dwarf, July 7, 1914, six weeks from time of planting.	1	23.6	402.4	2,595.7	37.4	241.5	241.5
	2	23.3	456.4	2,943.6	37.0	238.5	238.5
	3	23.4	363.9	2,347.1	32.2	207.4	207.4
	4	24.5	343.2	2,214.0	31.9	205.4	205.4
	5	25.8	397.3	2,562.6	32.7	211.2	211.2
Average.....		24.1	392.6	2,532.5	34.2	220.8	220.8
STAGE III.							
Corn, Pride of Saline, July 21, 1914, eight weeks after planting.	1	114.6	1,331.8	7,945.2	122.7	812.0	812.0
	2	137.2	1,431.7	9,152.9	127.1	820.7	820.7
	3	149.2	1,378.2	8,889.3	138.8	895.5	895.5
	4	140.1	1,266.6	8,169.8	124.8	802.2	802.2
	5	102.8	1,366.6	8,814.0	92.7	593.4	593.4
Average.....		128.7	1,333.4	8,600.4	122.3	788.3	788.3
Kafir, Blackhull, July 21, 1914, eight weeks from time of planting.	1	70.1	987.1	6,367.2	69.1	441.8	441.8
	2	75.9	955.7	6,238.7	57.8	374.5	374.5
	3	60.7	829.3	5,349.0	50.4	325.0	325.0
	4	68.3	893.9	5,760.2	52.6	341.4	341.4
	5	67.8	689.2	4,415.4	42.9	276.9	276.9
Average.....		68.5	871.0	5,631.3	59.4	383.3	383.3

<sup>1</sup>In this paper the term "leaf area" means the surface enclosed by the margins of the leaves. The total leaf surface exposed to the air therefore would be twice the leaf area.

TABLE VIII.—*Dry weight, leaf and sheath areas of corn, kafir, and milo at different stages of growth in 1914 at Garden City, Kans.—Continued*

Plant and period of growth.	Plant No.	Day weight of leaves and stems.	Leaf area.		Sheath area.	
		Gm.	Sq. in.	Sq. cm.	Sq. in.	Sq. cm.
STAGE III—continued.						
Milo, Dwarf, July 27, 1914, eight weeks from time of planting. Leaf growth completed	1	43.6	656.4	3,911.4	71.0	471.2
	2	54.4	664.6	4,280.8	49.8	295.8
	3	49.8	584.5	3,790.2	44.0	285.8
	4	57.0	593.8	3,872.0	51.7	346.49
Average	1	47.7	572.5	3,692.0	46.5	266.9
	2	51.4	605.1	3,922.9	51.4	312.4
STAGE IV.						
Corn, Pride of Saline, Aug. 4, 1914, ten weeks from time of planting. Leaf growth completed	1	167.4	2,273.5	8,215.0	192.1	1,239.1
	2	157.1	2,630.7	10,517.7	269.4	1,717.9
	3	175.0	1,374.6	8,543.7	210.9	1,360.6
	4	175.5	1,409.5	9,092.1	224.1	1,445.0
Average	1	165.5	1,544.4	5,929.3	82.9	530.2
	2	133.0	992.5	6,457.9	64.0	666.3
	3	113.9	917.0	5,914.9	102.5	667.9
	4	89.0	875.2	5,610.2	95.8	651.5
Average	1	121.2	891.3	5,748.8	93.2	606.5
	2	70.6	.....	.....	82.9	554.1
	3	54.6	.....	.....	67.6	415.9
	4	70.2	.....	.....	102.1	658.5
Average	1	69.3	.....	.....	89.4	576.6
	2	68.2	603.1	3,922.9	86.2	559.2

## SUMMARY

Plant and period of growth.	Height of plants.		Number of leaves. <sup>1</sup>	Dry weight of stem and leaves.	Leaf area. <sup>2</sup>		Sheath area.		Square centimeter of leaf area per gram of dry weight.
	Feet.	Cm.		Gm.	Sq. in.	Cm.	Sq. in.	Sq. cm.	
Stage I, June 24, 1914:									
Corn	1.5	45	4F 2P	12.5	241	1,553	18	115	215.0
Kafir	1.0	30	4F 4P	8.1	146	945	12	77	116.6
Milo	1.0	30	4F 4P	6.5	141	909	9	63	139.8
Stage II, July 7, 1914:									
Corn	2.5	75	6F 6P	51	841	5,344	68	440	102.8
Kafir	1.5	45	6F 4P	29.8	408	2,635	35	226	89.3
Milo	2.0	60	6F 3P	25.1	324	2,532	34	220	100.0
Stage III, July 27, 1914:									
Corn	4	120	9F 1P	128.7	1,333	8,600	122	788	66.8
Kafir	2.5	75	7F 3P	68.5	873	5,611	59	381	86.2
Milo	2.5	75	9	51.4	603	3,902	51	332	75.9
Stage IV, August 4, 1914:									
Corn	6	180	14-15	178.5	1,409	9,092	224	1,445	50.0
Kafir	4	120	12-14	241.1	872	5,748	93	605	51.8
Milo	3	90	9-10	60.2	605	3,922	86	559	58.9

<sup>1</sup> F=Leaves fully unfolded; P=Leaves partially unfolded.<sup>2</sup> Leaf surface equals twice these figures.

Stage I.—The plants reached this stage one month from the time of planting. Each plant showed four fully and four partially unfolded leaves. The Dwarf milo and Blackhull kafir plants had reached a height of 1 foot, while the corn plants stood 1½ feet high (Pl. XLIII, fig. 1). The leaf areas at this stage measured 1,553, 945, and 909 sq. cm. for corn, Blackhull kafir, and Dwarf milo, respectively, while the sheath areas of

these plants taken in the same order amounted to 116, 77, and 63 sq. cm. It is seen at this stage that the leaf area of corn was 1.7 times that of Dwarf milo and 1.64 times that of the Blackhull kafir.

Stage II.—The corn plants at this time had a height of  $2\frac{3}{4}$  feet and possessed six fully and six partially unfolded leaves. The Blackhull kafir measured  $1\frac{1}{2}$  feet in height and showed six fully and four partially unfolded leaves, while the Dwarf milo stood 2 feet high and had six fully and three partially unfolded leaves.

The plants reached this condition six weeks from the date of planting (Pl. XLIII, fig. 2). At this time

the leaf area of the corn had increased to 5,424 sq. cm., while that of the Blackhull kafir and Dwarf milo measured 2,635 and 2,332 sq. cm., respectively, the leaf area of the corn having increased to twice that of the Dwarf milo or Blackhull kafir. The leaf area of the two sorghums increased at the same rate up to this stage. The sheaths of all three plants showed an area approximately three times larger than they did when examined in the first stage.

Stage III.—The corn stood 4 feet high and had nine fully and five partially unfolded leaves. Blackhull kafir and Dwarf milo had each reached a height of  $2\frac{3}{4}$  feet. The former had seven fully and three partially unfolded leaves, while the latter was in the "booting stage" and possessed nine fully grown leaves (Pl. XLIV, fig. 1). The Dwarf milo at this stage had reached its full leaf development and showed a leaf area of 3,902 sq. cm. The leaf area of the corn plant was 2.2 times this, or 8,600 sq. cm. The leaf area of

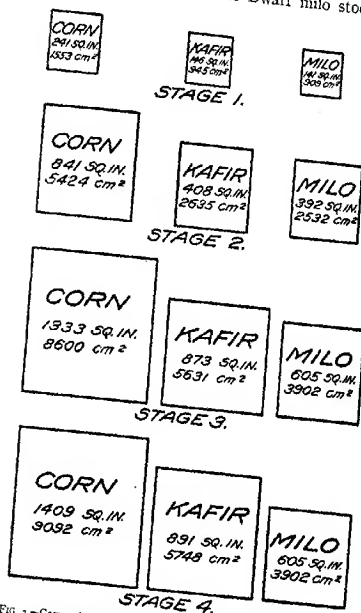


FIG. 2.—Comparison of the leaf areas of Pride of Saline corn, Blackhull kafir, and Dwarf milo at four stages of the growth of these plants during the season of 1914.

plants at this period were 8 weeks old. The corn stood 4 feet high and had nine fully and five partially unfolded leaves. Blackhull kafir and Dwarf milo had each reached a height of  $2\frac{3}{4}$  feet. The former had seven fully and three partially unfolded leaves, while the latter was in the "booting stage" and possessed nine fully grown leaves (Pl. XLIV, fig. 1). The Dwarf milo at this stage had reached its full leaf development and showed a leaf area of 3,902 sq. cm. The leaf area of the corn plant was 2.2 times this, or 8,600 sq. cm. The leaf area of

Blackhull kafir had increased to 5,631 sq. cm. and was 1.44 times the leaf extent of the Dwarf milo. The sheath area of the corn, Blackhull kafir, and Dwarf milo measured 788, 383, and 332 sq. cm., respectively.

Stage IV.—The plants at this stage had reached an age of 10 weeks and had completed their leaf development. The corn plants had from 14 to 15 leaves and the standard kafir from 12 to 14 leaves. The corn plants were 6 feet high, the standard kafir 4 feet high, while the Dwarf milo had reached a height of 3 feet (Pl. XLIV, fig 2). The leaf area of the corn plant at maturity was 9,092 sq. cm., an area 2.3 times that of the mature Dwarf milo, and 1.53 times that of the Blackhull kafir. The sheath area of these three plants was 1,445, 606, and 556 sq. cm., respectively, for corn, Blackhull kafir, and Dwarf milo.

#### SUMMARY

The root systems of Pride of Saline corn, Blackhull kafir, and Dwarf milo plants which were grown in alternate rows were isolated in the field at four stages of growth in 1914 and at three stages of growth in 1915. All told, the root systems of 33 plants were isolated and studied. It was found that for a given stage of growth each

plant possessed the same number of primary roots and that the general extent of these roots in both a horizontal and vertical direction was the same for all three plants. The maximum depth of root penetration for mature Dwarf milo, Blackhull kafir, and corn was found to be 6 feet for both the years 1914 and 1915. It was found that Blackhull kafir and Dwarf milo possessed approximately twice as many secondary roots per unit of primary root as did the corn plant. This is true not only for both years but also for all stages of the root systems examined. Both primary and secondary roots of the sorghums were found to be more fibrous than those of the corn plant.

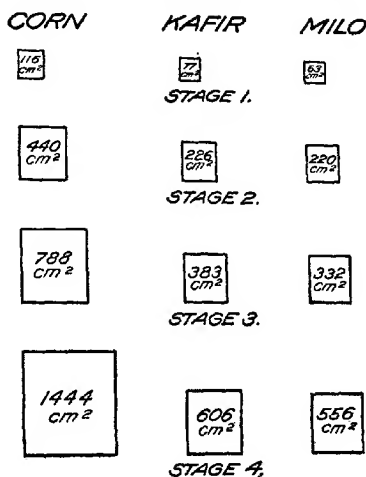


FIG. 3.—A graphic illustration of the sheath areas of Pride of Saline corn, Blackhull kafir, and Dwarf milo at four stages of the growth of these plants during the season of 1914.

The relation of the weight of the dry matter of the aerial portions of mature plants to the weight of the roots was determined in 1914 for 36 Dwarf milo plants, 30 Blackhull kafir plants, and 12 corn plants. In 1915 the same determinations were made for 24 Dwarf milo plants, 14 Dwarf Blackhull kafir plants, 23 Blackhull kafir plants, and 24 corn plants.

The average ratio of the dry weight of the grain, stem, and leaves of standard kafir to the dry weight of the roots was found to be 15 and 14.9 for the years 1914 and 1915, respectively, while the dry weight of the stem and leaves of the same plant was on the average 10.9 times that of the root weight in 1914 and 10.1 times the root weight in 1915. The ratio of the dry weight of the stem, leaves, and grain of Dwarf milo to the weight of the roots was found to be as 15.7 to 1 in 1914, and as 15 to 1 in 1915, and the weight of the stem and leaves of the same plants was 9.6 and 8 times, respectively, the weight of the roots in 1914 and 1915. The weight of the stem and leaves of Pride of Saline corn was 9.6 times the root weight in 1914, while in 1915 the weight of the stem and leaves of the corn was 7.8 times the weight of the root system. The aerial parts of Dwarf Blackhull kafir examined in 1915 showed a weight 15.7 times that of the roots, while the weight of the stem and leaves amounted to 8.9 times the weight of the underground portion.

The results of the experiments for the two years in regard to the soil-moisture content and depth of root penetration seem to show that under the conditions of this experiment very little, if any, depletion of soil moisture took place below the depth of root penetration.

The average leaf areas of five representative plants of corn, Blackhull kafir, and Dwarf milo were obtained at stages when the plants were 4, 6, 8, and 10 weeks of age. The last stage examined showed that the plants had completed their full-leaf development. In all the stages of growth the corn plant was found to have the greatest leaf area. Taking the stages of growth in order, one finds that the leaf area of the corn plant was 1.7, 2.0, 2.2, and 2.3 times the leaf area of Dwarf milo and 1.6, 1.9, 1.5, and 1.5 times that of Blackhull kafir.

In comparing the plants of Dwarf milo, Blackhull kafir, and Pride of Saline corn, it will be seen that in all stages of their growth these two sorghum plants have a primary root system that is just as extensive as that of the corn plant. In addition, the Dwarf milo and Blackhull kafir possess twice as many secondary roots as the corn at any stage of its growth. The leaf area of the corn plant at all stages of its growth is approximately twice as great as that of the Dwarf milo and never less than 1.5 times that of Blackhull kafir.

It is apparent, therefore, that the Dwarf milo and Blackhull kafir plants would have the advantage over the corn plant under any climatic condition that would tend to bring about a loss of water from these plants.

The two sorghums have, in the first place, as compared to the corn plant, only one-half the leaf surface exposed for the evaporation of water; and in the second place they have a root system which, judging from the number of secondary roots, would be twice as efficient in the absorption of water from the soil.

# LITERATURE CITED

- (1) BRIGGS, L. J., and SHANTZ, H. L.  
1912. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, 77 p., 9 fig., 2 pl.
- (2) ———  
1913. The water requirement of plants. I.—Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, 49 p., 11 pl.
- (3) HELLRIEGEL, Hermann.  
1883. Beiträge zu den naturwissenschaftlichen Grundlagen des Ackerbaus . . . Abschnitt 2.—Wurzel und Bodenvolumen. p. 118-280, 7 fig.
- (4) KIESSELBACH, T. A.  
1910. Transpiration experiments with the corn plant. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt. [1909], p. 125-139, 2 fig.
- (5) KING, F. H.  
1893. Natural distribution of roots in field soils. *In* Wis. Agr. Exp. Sta. 9th Ann. Rpt. [1891] 92, p. 112-120, fig. 9-17.
- (6) ROTMISTROV, V. G.  
1909. Root-Systems of Cultivated Plants of One Year's Growth. 57 p., illus. Odessa.
- (7) SCHULZE, B.  
1914? Studien über die Bewurzelung unserer Kulturpflanzen. *In* Festschrift, 50. Jubiläum Agr. Chem. Versuchs. u. Kontroll.-Stat., Breslau, p. 67-95, 10 pl.
- (8) SEELHORST, C. VON, and FRECKMANN, W.  
1903. Der Einfluss der Wassergehaltes des Bodens auf die Ernten und die Ausbildung verschiedener Getreide-Varietäten. *In* Jour. Landw., Bd. 51, Heft 3, p. 253-269.
- (9) TEN EYCK, A. M.  
1899. A study of the root systems of wheat, oats, flax, corn, potatoes and sugar beets and the soil in which they grew. N. Dak. Agr. Exp. Sta. Bul. 36, p. 333-362.
- (10) ———  
1900. A study of the root systems of cultivated plants grown as farm crops. N. Dak. Agr. Exp. Sta. Bul. 43, p. 533-550, 15 fig.
- (11) ———  
1904. The roots of plants. Kan. Agr. Exp. Sta. Bul. 127, p. 199-252, 1 fig., 26 pl.
- (12) TUCKER, M., and SEELHORST, C. VON.  
1898. Der Einfluss, welchen der Wassergehalt und der Reichthum des Bodens auf die Ausbildung der Wurzeln und der oberirdischen Organe der Haferpflanze ausüben. *In* Jour. Landw., Bd. 46, Heft 1, p. 52-63.

PLATE XXXVIII

Fig. 1.—Method used in isolating root systems in the field. View of two soil prisms ready for washing. The trenches here shown are 3 feet wide, 12 feet long, and 6 feet deep.

Fig. 2.—Method used in isolating root systems. This figure shows the method of placing the cross wires through the soil block.

Fig. 3.—Method of washing used in the isolation of the root systems. The trench was partially filled with water, which was continuously pumped upon the prism of soil by means of a pitcher pump.



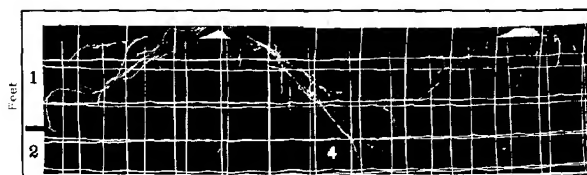
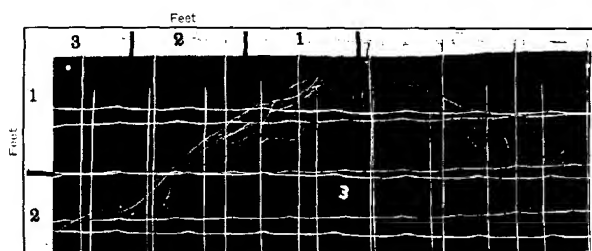
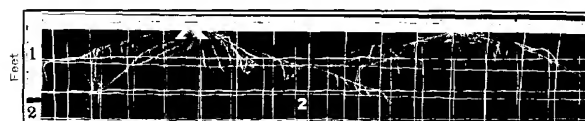
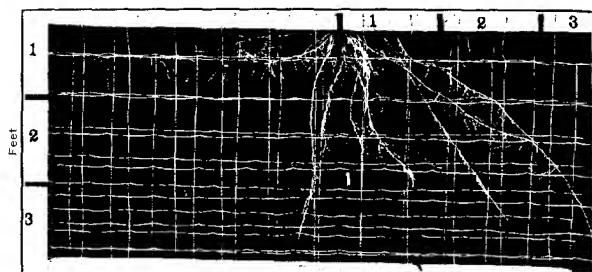


PLATE XXXIX

Fig. 1.—Root system of a corn plant that had reached a height of 3 feet 6 inches. Seed planted May 23, 1914. Root system isolated on July 17, 1914. Greatest depth of root penetration, 3 feet. Greatest lateral extent of the roots, 3 feet 6 inches.

Fig. 2.—Root systems of two corn plants with a height of 1 foot 6 inches. Seed planted on May 26, 1915. Root systems obtained on July 10, 1915. Greatest depth of roots, 1 foot 3 inches. Greatest lateral extent of roots, 2 feet 10 inches.

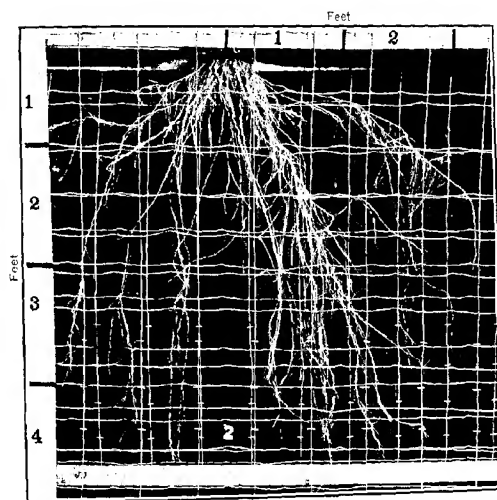
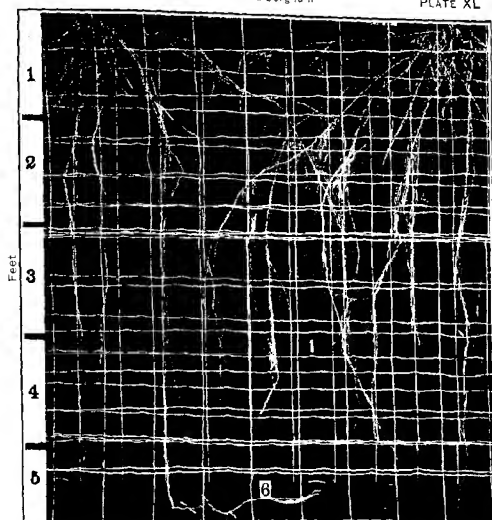
Fig. 3.—Root system of a Dwarf milo plant at the age of 4 weeks. Seed planted on May 23, 1914. Root system obtained on June 24, 1914. Plant stood 1 foot high. Greatest depth of root penetration, 1 foot 6 inches. Greatest lateral extent of roots, 3 feet.

Fig. 4.—Root systems of two Blackhull kafir plants 1 foot in height. Seed planted on May 26, 1915. Root systems isolated on July 10, 1915. Greatest depth of root penetration, 1 foot 6 inches. Greatest lateral extent of roots, 2 feet.

#### PLATE XL

Fig. 1.—Root systems of two mature corn plants. These plants stood 6 feet high, and the grain was in the glazed condition. Seed planted on May 23, 1914. Root systems obtained on August 25, 1914. Greatest lateral extent of the roots, 3 feet. Greatest depth of root penetration, 6 feet. The lower portion of the root cage is not shown here, but the roots which penetrated the sixth foot are shown in a horizontal position at the bottom of the cage.

Fig. 2.—Root system of a corn plant at the time of "shooting." Height of plant, 5 feet 6 inches. Seed planted on May 23, 1914. Root system obtained on August 7, 1915. Greatest depth of root penetration, 4 feet. Greatest lateral extent of the roots, 2 feet 6 inches.



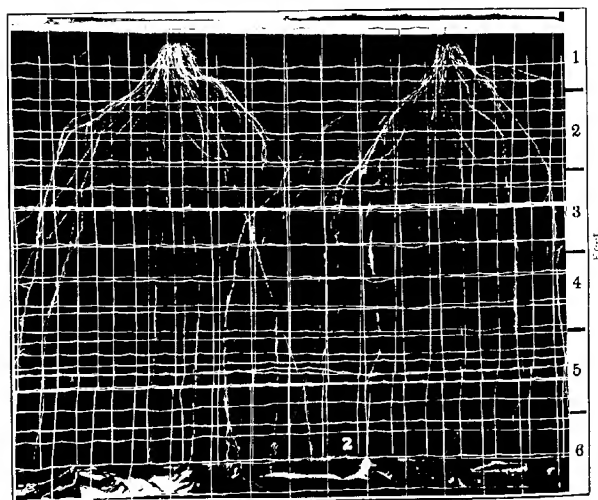
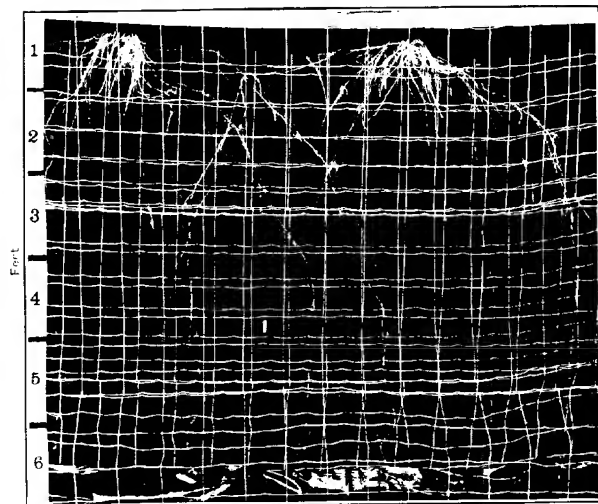


PLATE XLI

Fig. 1.—Root systems of two Blackhull kafir plants at the time they had reached a height of 6 feet and were blooming. Seed planted on May 26, 1915. Root systems isolated on September 3, 1915. Greatest depth of root penetration, 6 feet. Greatest lateral extent of the roots, 3 feet 8 inches.

Fig. 2.—Root system of two Dwarf milo plants at the time the seed was in the milk stage. The plants stood 3 feet 6 inches high. Seed planted on May 26, 1915. Root systems isolated on September 3, 1915. Greatest vertical penetration of the roots, 6 feet. Greatest lateral extent of the roots, 3 feet 8 inches.

#### PLATE XLII

Fig. 1.--Portion of a primary root of Pride of Saline corn, showing the number and relative size of the secondary roots. Both the primary and secondary roots of the corn are larger than those of the Dwarf milo or standard kafir.

Fig. 2.--Portions of the primary roots of Blackhull kafir, showing the number and relative size of the secondary roots. Both the primary and secondary roots of Dwarf milo and Blackhull kafir are smaller and more fibrous than those of the corn. The number of secondary roots per unit of length of primary root is twice as great for Blackhull kafir and Dwarf milo as for the corn.

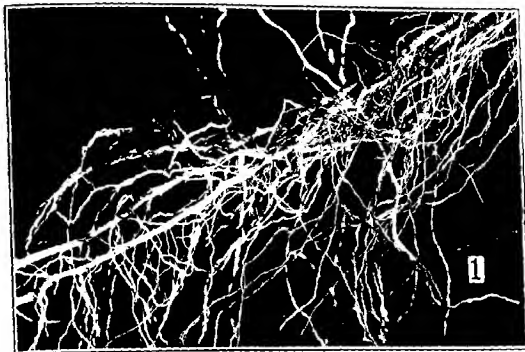


Figure 1. Root system of corn.

Figure 2. Root system of sorghum.



PLATE XLIII

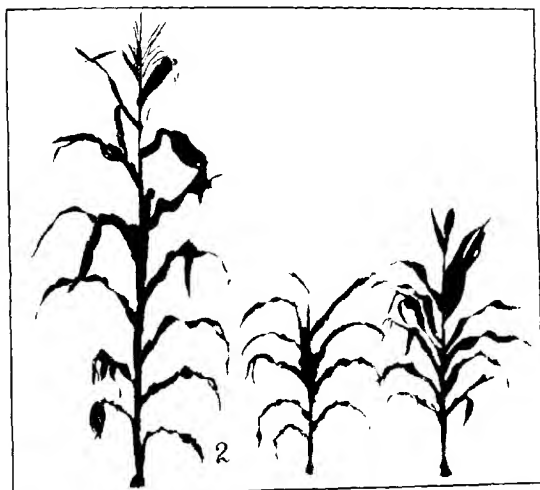
Fig. 1.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 4 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on June 24, 1914.

Fig. 2.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 6 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on July 7, 1914.

PLATE XLIV

Fig. 1.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 8 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on July 21, 1914.

Fig. 2.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 10 weeks of age. At this time the plants have completed their leaf development. Seed planted on May 23, 1914. Leaf areas determined on August 4, 1914.





## PRODUCTION OF CLEAR AND STERILIZED ANTI-HOG-CHOLERA SERUM

[PRELIMINARY PAPER]

By M. DORSET, *Chief*, and R. R. HENLEY, *Chemist, Biochemic Division, Bureau of  
Animal Industry*

### INTRODUCTION

In the United States the anti-hog-cholera serum of commerce for the most part consists of the defibrinated blood of hyperimmunized hogs. The red corpuscles contained in such commercial serum are not only devoid of protective qualities but are objectionable for a number of reasons. The practice of using the defibrinated hog's blood was adopted because of the difficulty experienced in separating completely the clear serum from the fibrin and the blood corpuscles.

Hog blood, when allowed to undergo spontaneous coagulation, ordinarily yields but a small proportion of clear serum. In practice not more than 30 or 35 per cent can be secured, the remainder of the serum being held firmly within the large clot. If, instead of allowing the blood to clot spontaneously, immediate defibrination be practiced, a yield of defibrinated blood varying from 90 to 95 per cent may usually be obtained. This defibrinated blood contains all of the antibodies present in the blood when drawn, whereas, if the blood is allowed to coagulate and the separated clear serum alone is used, there must be a large loss of antibodies, because part of the serum is held back in the clot.

The occurrence of the foot-and-mouth disease in the United States and the accidental infection of certain lots of hog-cholera serum and virus with this disease have demonstrated the urgent need for some method of treating these products which will serve to remove the possibility of either of them being a medium for its dissemination. In order to insure the freedom of hog-cholera serum from the virus of the foot-and-mouth disease, it is not sufficient merely to filter the product through bacteria-proof filters, because the virus of this disease itself is known to pass through bacteria-proof filters. It is likewise known that the virus of the foot-and-mouth disease is more or less resistant to the preservatives which are commonly used and which are suitable for the preservation of serum. There seems to be, therefore, only one means by which the serum may be sterilized in so far as the virus of the foot-and-mouth disease is concerned, and that is by the application of heat. The best European authorities state that this virus is killed when heated at a temperature of 50° C. for 12 hours. It also seems

well established that the virus is killed by 5 minutes' exposure to a temperature of 60°.

Experimental work has shown that defibrinated hog-cholera-immune blood may be heated to 50° C. for 12 hours without destroying the antibodies and without materially altering the physical character of the defibrinated blood. Heating to higher temperatures—60°, for example—results in more or less complete coagulation of the defibrinated blood, and therefore in the destruction of the serum in so far as its commercial worth is concerned. While heating at 50° for 12 hours might appear to be satisfactory, in practice it would be difficult and expensive to carry out such a process.

Experiments with clear serum, separated from the red cells, have shown that, unlike the defibrinated blood, which coagulates at 60°, the serum, separated from the red blood cells, withstands heating at 60° for 30 minutes without alteration of its physical characters and without noticeable impairment of its antitoxic power.

With the above facts in mind, renewed efforts have been made to devise a cheap and simple process for preparing hog-cholera antitoxin in the form of a clear serum free from the red blood corpuscles and from corpuscular debris.

#### PREPARATION OF THE SERUM

If ordinary defibrinated hog's blood be subjected to centrifugalization, there may be secured ordinarily about 50 per cent of serum. The time required will naturally depend to a large extent upon the precipitating force developed by the centrifuge. We have found that a force equivalent to approximately 1,700 times gravity serves to attain this result in from 20 to 30 minutes. The serum which separates is usually cloudy, and, owing to the fact that the red blood corpuscles are not firmly packed, it is impossible to remove all of the serum without at the same time carrying over some of the red cells. Therefore, simple centrifugalization has not seemed practicable for the following reasons: (1) Antibodies are lost because of inability to separate all of the serum from the corpuscles, (2) the serum secured is generally not clear, and (3) the removal of the serum from the cells is a difficult and tedious procedure.

In endeavoring to overcome the difficulties enumerated above, we have used extracts of the seed of different varieties of the common garden bean (*Phaseolus multiflorus* and *P. vulgaris*). Extracts of these beans are known to possess the property of agglutinating the red corpuscles of hog's blood, and they are said to be nontoxic.<sup>1</sup> Our own experience has shown that, although the extracts<sup>2</sup> exert no general systemic effect upon rabbits, guinea pigs, or hogs, certain varieties of these beans do yield extracts which act as intense local irritants, resulting, in guinea pigs

<sup>1</sup> Mendel, L. B. Observations on vegetable hæmagglutinins. *In Arch. Fisiol.*, v. 7, p. 168-177. 1909.

<sup>2</sup> Extracts made with water or normal salt solution.

at least, in swelling, followed by necrosis of tissue and the formation of suppurating abscesses at the sites of injection. The extracts of the scarlet runner bean (*P. multiflorus*) and of the pink kidney bean (*P. vulgaris*) are both intensely irritating, while extracts of the common white navy bean (*P. vulgaris*) are entirely lacking in this irritating property. While both the scarlet runner and the kidney bean are very powerful agglutinants, they have been rejected, at least temporarily, and extracts of the common white navy bean have been used exclusively in our later work.

Very minute amounts of the extracts of the navy bean serve to agglutinate large quantities of defibrinated hog's blood; and when such agglutinated blood is centrifugalized, the red cells pack together and form a rather stiff jelly-like mass in the tube. With a precipitating force of about 1,700 times gravity about 50 per cent of serum may be separated in 15 minutes. The serum is clear and may be readily poured from the tube.

In order to secure a greater yield of serum and a more firmly packed clot of red corpuscles, we find that the addition of a small quantity of sodium chlorid is very effective. The addition of 1 per cent of sodium chlorid to defibrinated hog's blood after agglutination from the addition of bean extract has begun will increase the yield of serum from 50 per cent without the salt to 70 per cent when the salt is added.

Considerable experimental work has led to the adoption of certain conditions of work as being most favorable to the production of the maximum amount of clear serum from defibrinated hog's blood. While experience may later show that some changes in procedure are desirable, it seems best to describe here the exact method, which is now being applied in these laboratories, of producing a clear sterile serum, heated to avoid the possibility of foot-and-mouth disease infection.

**PREPARATION OF BEAN EXTRACT.**—One hundred gm. of coarsely ground white navy beans are allowed to soak for one hour in 500 c. c. of distilled water, with occasional stirring. The pulp is strained through cheesecloth or cotton and mixed with powdered kieselguhr and filtered until clear. A filter of paper pulp mixed with some kieselguhr has been found to be efficient. The clear filtered extract is passed through a bacteria-proof filter of infusorial earth.

**PREPARATION OF DEFIBRINATED BLOOD FOR CENTRIFUGALIZING.**—To each 100 c. c. of the cool defibrinated blood add 1 c. c. of the sterile bean extract and stir to secure a uniform mixture. Allow the mixture to stand until agglutination is clearly evident. This can be determined by examining a small amount in a glass or tube. Agglutination is usually apparent within five minutes after adding the bean extract. There should then be added 1 gm. of finely powdered sodium chlorid. The salt is stirred in until dissolved, and the mixture of defibrinated blood, bean extract, and salt is allowed to stand for about 15 minutes.

**CENTRIFUGALIZING.**—The defibrinated blood mixture is placed in suitable containers, preferably somewhat elongated, and rotated in a centrifuge for 15 minutes at a speed sufficient to produce in the cups a precipitating force equal to approximately 1,700 times gravity. At the end of this period the serum may be poured from the cups into suitable containers.

**HEATING THE SERUM.**—The clear serum obtained by centrifugalizing is placed in a container which is surrounded by a jacket of water. The temperature of the water in the outer jacket at the beginning of the heating should not exceed 63° C. The serum in the inner container is slowly stirred during the heating process, the temperature of the outer jacket being maintained between 61° and 62°. A thermometer should be kept constantly in the serum and care should be taken to see that the temperature of the serum, once it has reached 60° C., does not fall below that point and that it does not rise materially above it.<sup>1</sup> Continuous heating for 30 minutes at 60° C. is required. Upon the completion of the heating, the serum should be rapidly cooled. After cooling, 1 part of a 5 per cent solution of phenol should be added to 9 parts of the serum.

**FILTERING THE SERUM.**—After the phenol has been added a slight precipitate may at times form in the serum; therefore it is desirable to allow several days to elapse between the addition of the phenol and the final filtration through infusorial earth.

#### EXPERIMENTAL RESULTS

To illustrate the yield of clear serum obtained by the application of the described method to the preparation of anti-hog-cholera serum, there is given in Table I a statement of the yield of clear serum obtained from three different lots of defibrinated immune blood and one lot of defibrinated hog-cholera virus.

TABLE I.—Yield of clear serum from defibrinated anti-hog-cholera serum and virus under a precipitating force of 1,700 times gravity applied for 12 minutes

Blood.	Bean extract added.	Sodium chlorid added.	Serum yield.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Hog-cholera serum from defibrinated immune blood 3895.....	None.	None.	47½
Do.....	1	1	49
Do.....	1	1	70
Do.....	1	1	70
Serum from defibrinated immune blood 3866 and 2105.....	1	1	74
Serum from defibrinated immune blood 2106.....	1	1	70
Serum from defibrinated hog-cholera virus 377 and 379.....	1	1	78

Table II gives the results of potency tests of one lot of serum prepared by use of the bean and sodium chlorid mixture. As will be seen, a test was made of the whole defibrinated blood, of the clear serum separated

<sup>1</sup> Thermometers used should be standardized, and the temperature of the serum should not be allowed to exceed 60.5° C.

from such defibrinated blood by the use of bean extract and sodium chlorid, and of the cell residues from which the clear serum was removed. In preparing the cells for injection they were taken up in distilled water and made to a volume corresponding to the volume of defibrinated blood from which they were derived. Thus hog 2143 received all of the cell residue from 200 c. c. of defibrinated blood and hog 2150 received all of the cell material from 100 c. c. of defibrinated blood. The serum which was obtained from the defibrinated blood was used to inoculate hogs 2155 to 2158, inclusive.

TABLE II.—Test of serum separated by use of bean extract and sodium chlorid in 1916<sup>a</sup>

Hog No.	Weight.	Date inoculated.	Protective material injected.	Quantity of protective material injected.	Quantity of virus.	Results.	Date died.
	<i>Pounds.</i>			<i>C. c.</i>	<i>C. c.</i>		
2143	70	Mar. 24	Phenolized defibrinated blood 3895.	20	2	Remained normal throughout test.	
2144	65	do.	Phenolized defibrinated blood 3895 (unwashed).	10	1	do.	
2149	70	do.	Cells from defibrinated blood 3895.	200	2	Injured in fighting Mar. 27; off feed Mar. 28 to Apr. 4. Very slight hemorrhagic lesions.	Apr. 4
2150	65	do.	do.	100	2	Went off feed Mar. 27; very sick Mar. 28 to Apr. 21. Extensive hemorrhagic lesions.	Apr. 21
2155	60	do.	Clear serum from defibrinated blood 3895 heated.	16	2	Remained normal throughout test.	
2156	65	do.	do.	16	2	do.	
2157	50	do.	do.	8	2	do.	
2158	55	do.	do.	8	2	Well-marked lesions of hog cholera on post-mortem examination.	Apr. 12
2159	45	do.	Control.		2	Extensive lesions of hog cholera on post-mortem examination.	Apr. 21
2164	50	do.	do.		2		

<sup>a</sup> No inflammation or swelling at point of injection on any pigs in this test. Thriftiness of pigs remaining normal not impaired.

From the fact that both of the pigs injected with the cell material contracted hog cholera and died it seems clear that, in this experiment at least, the amount of antibodies left behind with the cells was negligible.

The bean-extract-sodium-chlorid method of separating the corpuscles from defibrinated hogs' blood has been applied repeatedly in these laboratories and always with success. There seems to be no reason why the process should not be entirely satisfactory for use in the practical production of anti-hog-cholera serum. There appears to be little or no loss in antibodies; the serum secured is generally clear; and it may be removed from the agglutinated cells easily by pouring from the cups. The method also would seem to tend toward a certain concentration of

the antibodies of the blood, and it is also to be recommended on account of the fact that it results in a large yield of serum.

The fact that this serum may be heated for half an hour at 60° C. without noticeable impairment of its potency is of much practical importance because there is thus afforded a ready means for safeguarding it against infection with the virus of the foot-and-mouth disease.

Anyone contemplating the practical application of the process is urged, at the beginning at least, to follow the method described herein, and to use only the common white navy bean for preparing the bean extract. It is hoped that the method will soon be adopted on a large scale by commercial producers of serum.

ADDITIONAL COPIES  
OF THIS PUBLICATION MAY BE PROCURED FROM  
THE SUPERINTENDENT OF DOCUMENTS  
GOVERNMENT PRINTING OFFICE  
WASHINGTON, D. C.

AT  
15 CENTS PER COPY  
SUBSCRIPTION PRICE, \$3.00 PER YEAR



